REVIEW

Tobacco Smoke Carcinogens and Lung Cancer

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The complexity of tobacco smoke leads to some confusion about the mechanisms by which it causes lung cancer. Among the multiple components of tobacco smoke, 20 carcinogens convincingly cause lung tumors in laboratory animals or humans and are, therefore, likely to be involved in lung cancer induction. Of these, polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone are likely to play major roles. This review focuses on carcinogens in tobacco smoke as a means of simplifying and clarifying the relevant information that provides a mechanistic framework linking nicotine addiction with lung cancer through exposure to such compounds. Included is a discussion of the mechanisms by which tobacco smoke carcinogens interact with DNA and cause genetic changes—mechanisms that are reasonably well understood—and the less well defined relationship between exposure to specific tobacco smoke carcinogens and mutations in oncogenes and tumor suppressor genes. Molecular epidemiologic studies of gene–carcinogen interactions and lung cancer—an approach that has not yet reached its full potential—are also discussed, as are inhalation studies of tobacco smoke in laboratory animals and the potential role of free radicals and oxidative damage in tobacco-associated carcinogenesis. By focusing in this review on several important carcinogens in tobacco smoke, the complexities in understanding tobacco-induced cancer can be reduced, and new approaches for lung cancer prevention can be envisioned. [J Natl Cancer Inst 1999;91:1194–1210]

Lung cancer continues to be the leading cause of cancer death in both men and women in the United States, with more than 158 900 deaths expected in 1999 *(1)*. Worldwide, lung cancer kills over one million people each year *(2)*. Extensive prospective epidemiologic data clearly establish cigarette smoking as the major cause of lung cancer *(3)*. It is estimated that about 90% of male lung cancer deaths and 75%–80% of female lung cancer deaths in the United States each year are caused by smoking *(4,5)*. The risk of lung cancer diminishes after smoking cessation, but not during the first 5 years, and the relative risk never returns to that of a nonsmoker *(3)*. In spite of the rising antitobacco sentiment in the United States and improvements in smoking cessation methods, approximately 25% of the U.S. adult population, about 47 million people, continues to smoke cigarettes *(6)*. Although the percentage of adult smokers decreased following the first Surgeon General's report *(7)*, from 42% in 1965 to 25% in 1990, there has been virtually no change since then, suggesting that we may have reached a hard-core population of smokers *(8)*. Approximately five hundred billion cigarettes were sold in the United States in 1995 *(9)*. There are one billion cigarette smokers worldwide, one third of whom live in China, where a major epidemic of lung cancer is predicted *(10,11)*. Although the argument for further tobacco control and improved cessation strategies is powerful, the numbers tell us that the utopian goal of a smoke-free society is still distant *(6)*. Moreover, exposure to environmental tobacco smoke (ETS) is widely accepted as a cause of lung cancer, although the risk is far lower than that of smoking and can be difficult to demonstrate, even in large studies *(12–16)*. An understanding of mechanisms of tobacco-induced lung cancer will lead to new strategies for decreasing lung cancer risk, for identifying highly susceptible individuals, and for developing innovative techniques for early detection.

Even in the writings of distinguished scientists with great expertise in cancer causes and mechanisms, one can read statements such as: "The carcinogenic mechanisms of tobacco smoking are not well understood" *(17)*. This review will attempt to provide the generally informed cancer scientist with a distillation of mechanistic information on the subject of tobacco smoke carcinogens and lung cancer and to convince the reader that we know a great deal about the mechanisms by which these carcinogens cause lung cancer. While it is true that we may never be able to map each detail of the complex process by which cigarette smoking causes lung cancer and that there is unlikely to be a single mechanism of tobacco carcinogenesis, there are general principles that have emerged from intensive research in the past four to five decades. The overall framework for discussing this information is illustrated in Fig. 1. Carcinogens form the link between nicotine addiction and lung cancer. Nicotine addiction is the reason that people continue to smoke *(18)*. While nicotine itself is not considered to be carcinogenic, each cigarette contains a mixture of carcinogens, including a small dose of polycyclic aromatic hydrocarbons (PAHs) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) among other lung carcinogens, tumor promoters, and co-carcinogens *(19,20)*. Carcinogens such as NNK and PAHs require metabolic activation to exert their carcinogenic effects; there are competing detoxification pathways, and the balance between metabolic activation and detoxification differs among individuals and will affect cancer risk.

We know a great deal about mechanisms of carcinogen metabolic activation and detoxification *(21–25)*. The metabolic activation process leads to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA, usually at guanine or adenine. There have been major advances in our understanding of DNA adduct structure and its consequences in the past two decades, and we now have a large amount of mechanistic information *(26,27)*. If DNA adducts escape cellular

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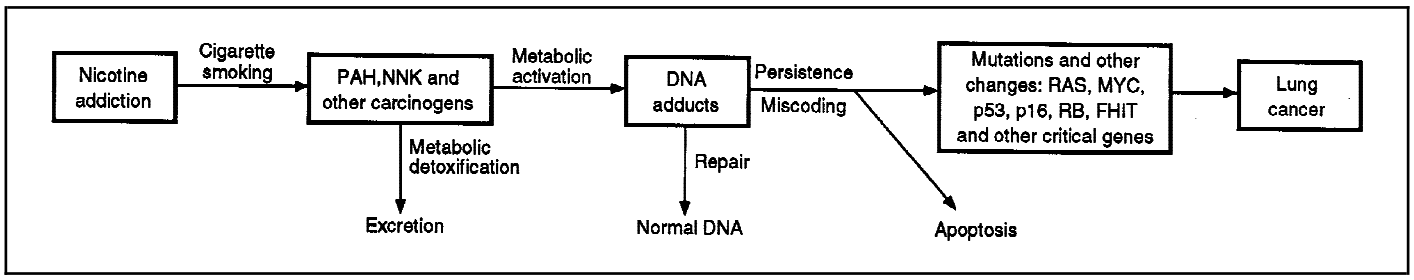

Fig. 1. Scheme linking nicotine addiction and lung cancer via tobacco smoke carcinogens and their induction of multiple mutations in critical genes. PAH = polycyclic aromatic hydrocarbons; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

repair mechanisms and persist, they may lead to miscoding, resulting in a permanent mutation. As a result of clever strategies that combine DNA adduct chemistry with the tools of molecular biology *(28)*, we know a great deal about the ways in which carcinogen DNA adducts cause mutations. Cells with damaged DNA may be removed by apoptosis, or programmed cell death *(29,30)*. If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to activation of the oncogene or deactivation of the tumor suppressor gene. Multiple events of this type lead to aberrant cells with loss of normal growth control and, ultimately, to lung cancer. While the sequence of events has not been as well defined as in colon cancer, there can be little doubt that these molecular changes are important *(29,30)*. There is now a large amount of data on mutations in the human KRAS and p53 (also known as TP53) genes in lung tumors from smokers, and attempts have been made to link these mutations to specific carcinogens in tobacco smoke *(30–36)*. Blocking any of the horizontal steps in Fig. 1 may lead to decreased lung cancer, even in people who continue to smoke.

This review will focus on tobacco smoke carcinogens and will consider certain aspects of the mechanistic pathway illustrated in Fig. 1. It will discuss pulmonary carcinogens in cigarette smoke, inhalation studies, and investigations of lung carcinogen uptake, metabolism, and DNA adduct formation in humans. It will also consider other mechanisms of DNA damage via free radicals and reactive oxygen species. It will discuss mutations in oncogenes and tumor suppressor genes and their possible relationship to specific carcinogens and molecular epidemiologic investigations of carcinogen–gene interactions. On the basis of these data, it will evaluate the role of specific cigarette smoke carcinogens and other factors as causes of lung cancer. A detailed account of other aspects of the molecular pathogenesis of lung cancer has recently been published *(30)*.

The goal of this review is to be illustrative rather than inclusive. Any of the topics mentioned in the previous paragraph would exceed the space limitations of this Journal if presented completely. This review used MEDLINE®, the International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans, the Survey of Compounds Which Have Been Tested for Carcinogenic Activity, and selected previous reviews and key references to identify references on specific topics.

Pulmonary Carcinogens in Cigarette Smoke

The mainstream smoke emerging from the mouthpiece of a cigarette is an aerosol containing about 10^{10} particles/mL *(20)*. About 95% of the smoke is made up of gases, chiefly nitrogen, oxygen, and carbon dioxide. In experiments, these vapor-phase components are separated from the particulate phase by a glass-fiber filter. The particulate phase contains at least 3500 compounds and most of the carcinogens *(20)*. The components of cigarette smoke and how they have changed over time have been reviewed previously *(19,20,37,38)*.

There are 55 carcinogens (Table 1, A) in cigarette smoke that have been evaluated by the International Agency for Research on Cancer (IARC) and for which there is "sufficient evidence for carcinogenicity" in either laboratory animals or humans *(19)*. Other carcinogens not evaluated by the IARC may also be present. For example, among the PAHs, multiple alkylated and high-molecular-weight compounds have been detected but are incompletely characterized with respect to their carcinogenicity *(39,40)*. Individual pulmonary carcinogens in cigarette smoke, selected from the classes of carcinogens in Table 1, A, are listed in Table 1, B. The 20 compounds included in this list have been found convincingly to induce lung tumors in at least one animal species and have been positively identified in cigarette smoke. The structures of the organic compounds are shown in Fig. 2.

Among the PAHs, benzo[*a*]pyrene (BaP) is the most extensively studied compound, and its ability to induce lung tumors upon local administration or inhalation is well documented *(41,42,62,63)*. When administered systemically, it causes lung tumors in mice, but not in rats *(41,42,64)*. In studies of lung tumor induction by implantation in rats, BaP is more carcinogenic than the benzofluoranthenes or indeno[1,2,3-*cd*]pyrene *(43)*. In analytic studies, it has often been used as a surrogate for other PAHs, and extensive data on its occurrence in cigarette smoke are available *(19,38)*. Thus, BaP is a potent lung carcinogen, the occurrence of which is well documented. The vast literature on BaP tends to distract attention from other PAHs. However, PAHs such as dibenz[*a*,*h*]anthracene, 5-methylchrysene, and dibenzo[*a*,*i*]pyrene are substantially stronger lung tumorigens than BaP in mice or hamsters, although the levels of these compounds in cigarette smoke are lower than those of BaP *(44,45)*. The presence in cigarette smoke of dibenzo[*a*,*l*]pyrene, a highly carcinogenic PAH, has not been confirmed.

Two aza-arenes, dibenz[*a*,*h*]acridine and 7H-dibenzo[*c*,*g*]-carbazole, are pulmonary tumorigens when tested by implantation in the rat lung and instillation in the hamster trachea, respectively *(46,47)*. The activity of dibenz[*a*,*h*]acridine is significantly less than that of BaP, while the activity of 7H-dibenzo[*c*,*g*]carbazole is greater than that of BaP. The levels of both compounds in cigarette smoke are relatively low.

Among the *N*-nitrosamines, *N*-nitrosodiethylamine is an effective pulmonary carcinogen in the hamster, but not in the rat *(48,49)*. Its levels in cigarette smoke are low compared with

Table 1, A. Summary of carcinogens in cigarette smoke*

Type	No. of compounds
Polycyclic aromatic hydrocarbons	10
Aza-arenes	3
N-Nitrosamines	7
Aromatic amines	3
Heterocyclic aromatic amines	8
Aldehydes	2
Miscellaneous organic compounds	15
Inorganic compounds	7
Total	55

Table 1, B. Pulmonary carcinogens in cigarette smoke†

Carcinogen class	Compound	Amount in mainstream cigarette smoke, ng/cigarette‡	Sidestream/ mainstream ratio§	Representative lung tumorigenicity in species‖	Reference No.‖
Polycyclic aromatic hydrocarbons	Benzo[*a*]pyrene	20–40	2.5–3.5	Mouse, rat, hamster	*(41,42)*
	Benzo[*b*]fluoranthane	4–22		Rat	*(41–43)*
	Benzo[*j*]fluoranthane	6–21		Rat	*(41–43)*
	Benzo[*k*]fluoranthane	6–12		Rat	*(41–43)*
	Dibenzo[*a,i*]pyrene	1.7–3.2		Hamster	*(41,42,44)*
	Indeno[1,2,3-*cd*]pyrene	4–20		Rat	*(41–43)*
	Dibenz[*a,h*]anthracene	4		Mouse	*(41,42,45)*
	5-Methylchrysene	0.6		Mouse	*(42,45)*
Asz-arenes	Dibenz[*a,h*]acridine	0.1		Rat	*(41,42,46)*
	7H-Dibenzo[*c,g*]carbazole	0.7		Hamster	*(41,42,47)*
N-Nitrosamines	*N*-Nitrosodiethylamine	ND–2.8	<40	Hamster	*(48,49)*
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	80–770	1–4	Mouse, rat, hamster	*(22,50)*
Miscellaneous organic compounds	1,3-Butadiene	$20–70 \times 10^3$		Mouse	*(51)*
	Ethyl carbamate	20–38		Mouse	*(52)*
Inorganic compounds	Nickel	0–510	13–30	Rat	*(53)*
	Chromium	0.2–500		Rat	*(53)*
	Cadmium	0–6670	7.2	Rat	*(54)*
	Polonium-210	0.03–1.0 pCi	1.0–4.0	Hamster	*(55–58)*
	Arsenic	0–1400		None¶	*(59)*
	Hydrazine	24–43		Mouse	*(60)*

*Adapted from *(19,20)*. Compounds for which there is "sufficient evidence for carcinogenicity" in either laboratory animals or humans, according to evaluations by the International Agency for Research on Cancer.

†Compounds from Table 1, A, for which there is convincing evidence of pulmonary tumorigenicity in at least one species.

‡Data from *(19,37);* all values in ng/cigarette except polonium-210; ND = not detectable.

§Data from *(61)*.

‖Studies in laboratory animals.

¶Epidemiologic studies indicate that inorganic arsenic compounds are skin or lung carcinogens in humans.

those of other carcinogens. The tobacco-specific *N*-nitrosamine NNK is a potent lung carcinogen in rats, mice, and hamsters *(22)*. It is the only compound in Table 1, B, that induces lung tumors systemically in all three commonly used rodent models. The organospecificity of NNK for the lung is remarkable; it induces tumors of the lung, mainly adenoma and adenocarcinoma, independent of the route of administration and in both susceptible and resistant strains of mice (Table 2) *(22)*. NNK has not been tested by local administration in the respiratory tract. The systemic administration of NNK to rats is a reproducible and robust method for the induction of lung tumors; dose–response data from two laboratories are summarized in Fig. 3 *(22)*. Cigarette smoke contains substantial amounts of NNK *(19,38,65–67)*, and the total dose experienced by a smoker in a lifetime of smoking is remarkably close to the lowest total dose shown to induce lung tumors in rats *(22)*. Levels of NNK and total PAHs in cigarette smoke are similar *(20)*.

The lung is one of the multiple sites of tumorigenesis by 1,3-butadiene in mice, but it is not a target in the rat *(51)*. 1,3-Butadiene is a component of the vapor phase of cigarette smoke, but in most inhalation studies the particulate phase shows more overall carcinogenic activity. Ethyl carbamate is a well-established pulmonary carcinogen in mice, but not in other species *(52)*. Nickel, chromium, cadmium, and arsenic are all present in tobacco, and a percentage of each is transferred to mainstream smoke; arsenic levels are substantially lower since discontinuation of its use as a pesticide in 1952 *(20,37,38)*. Metal carcinogenicity depends on the valence state and anion; these are poorly defined in many analytical studies of tobacco smoke. Thus, although some metals are effective pulmonary carcinogens, the role of metals in tobacco-induced lung cancer is murky. Levels of polonium-210 in tobacco smoke are not believed to be great enough to significantly impact lung cancer in smokers *(68)*. Hydrazine is an effective lung carcinogen in mice and has been detected in cigarette smoke in limited studies *(38,60)*.

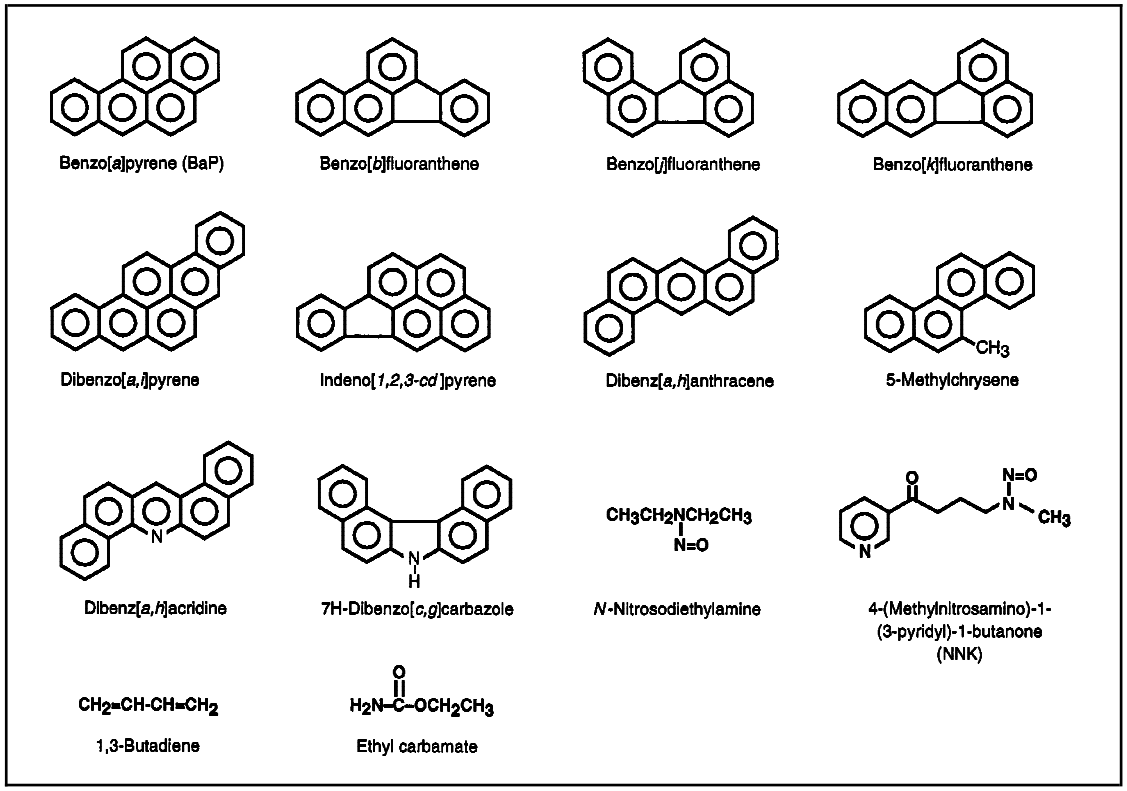

Fig. 2. Structures of organic pulmonary carcinogens in tobacco smoke.

Table 2. Induction of lung tumors by NNK [4-(methylnitrosamino)-1-[3-pyridyl)-1-butanone]*

Species and strain	Route†
Mouse	
A/J	i.p. gavage, p.o.
Sencar	Skin
BALB/c	Oral
Swiss	Oral, i.p.
$C3B6F_1$	i.p.
C3H/HeJ	i.p.
C57BL/6	i.p.
(A/J × TSG-p53) F_2	i.p.
F344 rat	s.c., p.o., oral swab, gavage, intravesicular
Syrian golden hamster	s.c., application to cheek pouch
Mink	s.c.

*Adapted from *(22)*.

†i.p. = intraperitoneal; p.o. = per os (i.e., orally via drinking water); and s.c. = subcutaneous.

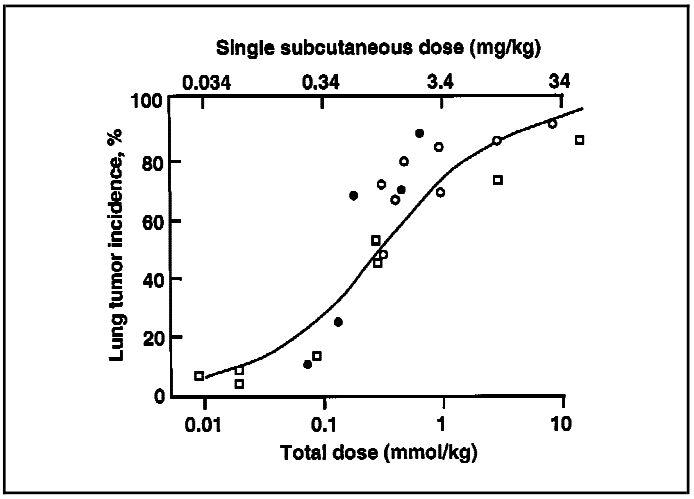

Fig. 3. Relationship between dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and lung tumor incidence in male F344 rats. Data were combined from protocols using subcutaneous (s.c.) injection, three times weekly for 20 weeks, in studies carried out by investigators at the National Institute of Environmental Health Sciences (□) or the American Health Foundation (○), or by administration in the drinking water (●) (American Health Foundation). Upper *x*-axis, single s.c. dose refers to the magnitude of one of the 60 doses used. Each symbol represents a group of 20–80 rats. From *(22)*.

The carcinogens listed in Table 1, B, are also found in ETS *(61)*. Sidestream smoke, the material released directly into the air from the burning tip of a cigarette plus that which diffuses through the cigarette paper, constitutes the major portion of ETS *(61)*. Some sidestream–mainstream ratios are presented in Table 1, B. While these ratios are generally greater than 1, dilution with ambient air is such that passive uptake will be far less than uptake in a smoker, and the risk for lung cancer is accordingly less *(69)*.

Cigarette smoke is also a tumor promoter *(38,70)*. The majority of the activity seems to be due to uncharacterized weakly acidic compounds. Substantial levels of cocarcinogens such as catechol are present in cigarette smoke *(70)*. Other cocarcinogens include methylcatechols, pyrogallol, decane, undecane, pyrene, benzo[*e*]pyrene, and fluoranthene. In addition, cigarette smoke contains high levels of acrolein, which is toxic to the pulmonary cilia, and other agents, such as nitrogen oxides, acetaldehyde, and formaldehyde, that could contribute indirectly to pulmonary carcinogenicity *(38)*.

Inhalation Studies of Cigarette Smoke

While extensive studies clearly document the carcinogenicity of certain cigarette smoke constituents, the results of inhalation studies of whole-cigarette smoke or its vapor and particulate

phases have been less consistent. Cigarette smoke inhalation studies through 1985 have been reviewed elsewhere *(38)*. There are a number of operational problems inherent in these experiments. The smoke must be delivered in a standardized fashion that has been accomplished with a variety of designs. Both whole-body exposure and nose-only designs have been used. Generally, a 2-second puff from a burning cigarette is diluted with air and forced into the chamber. Animals will undergo avoidance reactions and will not inhale the smoke the way humans do. Thus, the dose to the lung in animals will be less than that in humans. It will also be considerably less than in most experiments that examine the carcinogenicity of individual components of smoke. Other problems arise from the fact that rodents are obligatory nose breathers and their nasal passages are more complex than those of humans, thereby affecting the dynamics of particle deposition in the respiratory tract. The irritating and toxic properties of tobacco smoke create further difficulties.

Nevertheless, in experiments with Syrian golden hamsters, whole-cigarette smoke and its particulate phase consistently induce preneoplastic lesions and benign and malignant tumors of the larynx *(38)*. This model system has been widely applied and is the most reliable one for induction of tumors by inhalation of cigarette smoke. Tumors are observed in hamsters exposed to the particulate phase of smoke only. Results of experiments in rats and mice are inconsistent, while those of experiments in rabbits and dogs are equivocal. Studies published since 1985 describe inhalation experiments with mice *(71–74)*. Two studies are negative, but two others evaluating the activity of ETS in A/J mice show moderately positive results. In these studies *(73,74)*, increased lung tumor multiplicity is observed in mice exposed to ETS and then allowed a recovery period. It was concluded that the vapor phase of ETS is as tumorigenic as is full ETS and that the responsible agents are not NNK or BaP. These studies require confirmation. Further research is needed to identify the putative tumorigenic components of the vapor phase.

Pulmonary Carcinogens: Uptake, Metabolism, and Adduct Formation in Smokers

Carcinogens are enyzmatically transformed to a series of metabolites as the exposed organism attempts to convert them to forms that are more readily excreted. The initial steps are usually carried out by cytochrome P450 (P450) enzymes, encoded by the CYP family of genes, which oxygenate the substrate *(75)*. Other enzymes, such as lipoxygenases, cyclooxygenases, myeloperoxidase, and monoamine oxidases, may also be involved, but less frequently. The oxygenated intermediates formed in these initial reactions may undergo further transformations by glutathione *S*-transferases, uridine-5′-diphosphate-glucuronosyltransferases, sulfatases, and other enzymes *(76–78)*. Some of the metabolites produced by the P450s react with DNA or other macromolecules to form covalent binding products known as adducts. This is referred to as metabolic activation; other reactions are considered as detoxification pathways with respect to carcinogenesis. Metabolic pathways of BaP and NNK, representative pulmonary carcinogens in cigarette smoke, are outlined in Fig. 4 *(21,22,42,79–100)*. These have been extensively studied

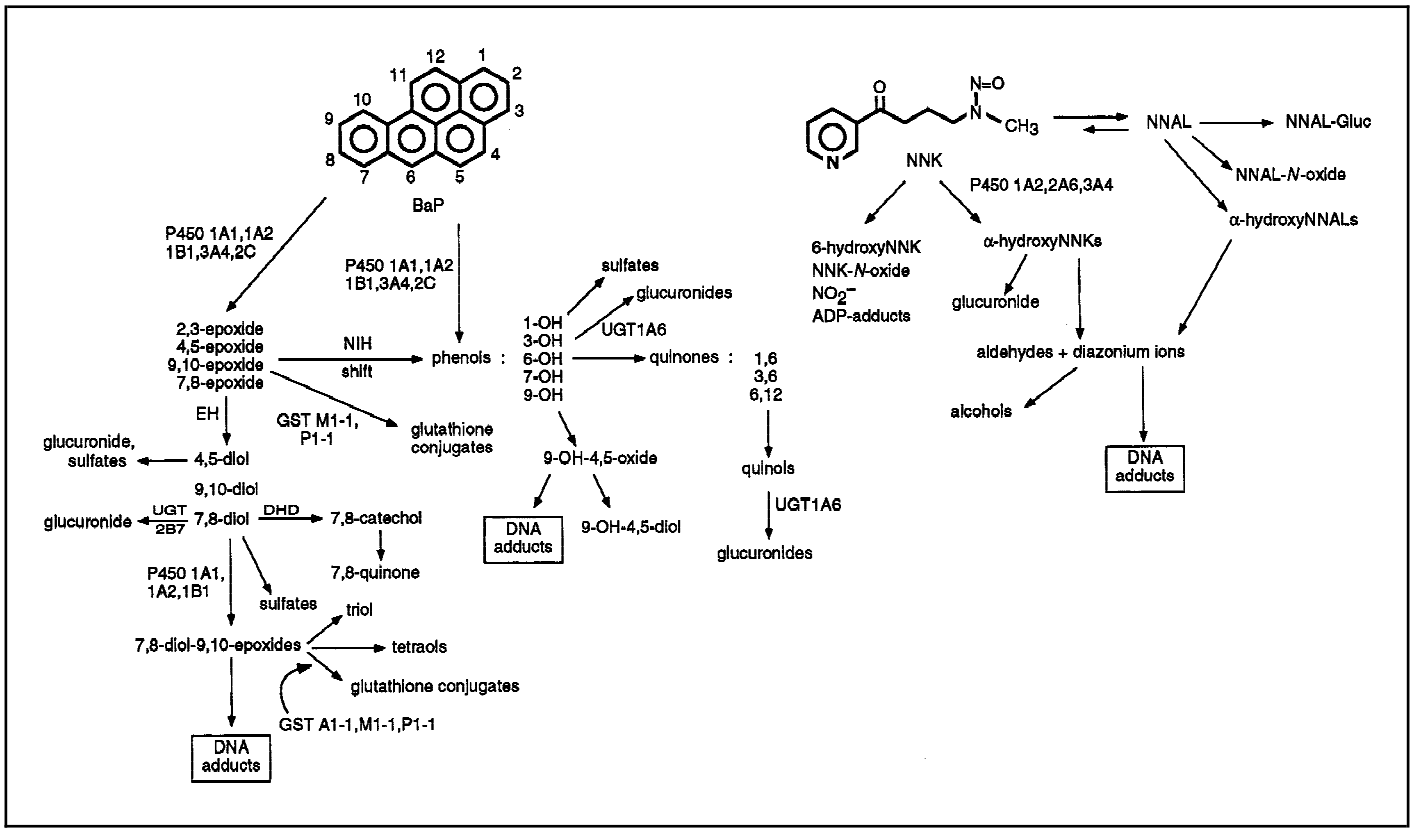

Fig. 4. Metabolic pathways of benzo[*a*]pyrene (BaP) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), modified from *(22,80,83)*. Some human enzymes involved in the various reactions are indicated *(79–100)*. EH = epoxide hydrolase; DHD = dihydrodiol dehydrogenase; UGT = UDP-glucuronosyl transferase; GST = glutathione *S*-transferase; P450 = cytochrome P450; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 1-OH, 3-OH = 1-hydroxy BaP, 3-hydroxy BaP, etc. Other abbreviations are defined in the text.

in rodent and human tissues. Multiple enzymes participate in many steps; some of the human forms involved are indicated in Fig. 4. The major metabolic activation pathway of BaP is conversion to its 7,8-diol-9,10-epoxides (BPDE); one of the four enantiomers is highly carcinogenic and reacts with DNA to form adducts with N^2 of deoxyguanosine. The major metabolic activation pathways of NNK and its main metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), occur by hydroxylation of the carbons adjacent to the *N*-nitroso group (α-hydroxylation), which leads to the formation of two types of DNA adducts: methyl adducts, such as 7-methyguanine or O^6-methylguanine, and pyridyloxobutyl adducts. O^6-Methylguanine plays a critical role in mouse lung tumorigenesis by NNK, while this adduct as well as pyridyloxobutyl DNA adducts are important in lung tumor induction by NNK in rats *(22)*.

Considerable information is available on pulmonary carcinogen metabolism *in vitro*, both in animal and in human tissues, but fewer studies have been carried out on uptake, metabolism, and adduct formation of cigarette smoke lung carcinogens in smokers. Various measures of cigarette smoke uptake in humans have been used, including exhaled carbon monoxide, carboxyhemoglobin, thiocyanate, and urinary mutagenicity *(38)*. However, the most specific and widely used biochemical marker is the nicotine metabolite cotinine *(38,101)*. While cotinine and other nicotine metabolites are excellent indicators of tobacco smoke constituent uptake by smokers, the NNK metabolites NNAL and its *O*-glucuronide (NNAL-Gluc) are excellent biomarkers of tobacco smoke lung carcinogen uptake *(22)*. NNAL is a potent pulmonary carcinogen like NNK, while NNAL-Gluc is a detoxified metabolite of NNK *(22)*. Since NNK is a tobacco-specific carcinogen, its metabolites NNAL and NNAL-Gluc are found only in the urine of individuals exposed to tobacco products. Urinary NNAL and NNAL-Gluc have been quantified in several studies of smokers and in nonsmokers exposed to ETS *(22,102–107)*. The ETS results demonstrate that uptake of NNAL-Gluc by nonsmokers is 1%–3% of that in smokers, consistent with the weaker epidemiologic evidence for a role of ETS, compared with mainstream cigarette smoke, as a cause of lung cancer *(69,107)*. Levels of cotinine plus cotinine-*N*-glucuronide in smokers' urine are correlated with urinary NNAL plus NNAL-Gluc (Fig. 5). Similar correlations are observed in passively exposed nonsmokers *(103,107)*.

Since NNAL is a potent lung carcinogen, but NNAL-Gluc is not carcinogenic, the ratio of NNAL-Gluc to NNAL has been suggested as a potential biomarker of susceptibility to lung cancer *(104)*. This ratio varies widely in smokers *(104)*. It is interesting that the NNAL-Gluc : NNAL ratio was significantly lower in black smokers than in white smokers, which suggests that poor detoxification potential may be one factor contributing to the higher incidence and mortality rates of lung cancer in blacks than in whites *(108)*. Related to this, two recent studies *(109,110)* have clearly demonstrated that serum cotinine levels are higher in blacks than in whites. This is postulated to result from higher nicotine intake per cigarette and slower clearance of cotinine in blacks.

BaP and benzo[*k*]fluoranthene have been detected in human lung; no differences between smokers and nonsmokers were noted *(111,112)*. 1-Hydroxypyrene and its glucuronide, urinary metabolites of the noncarcinogen pyrene, have been widely used as indicators of PAH uptake. 1-Hydroxypyrene levels in smokers are generally higher than those in nonsmokers *(113–115)*. Other studies *(116–122)* have examined PAHs in urine by reconversion of metabolites to the parent compounds or have detected specific PAH metabolites, including 3-hydroxy BaP and tetraols resulting from hydrolysis of BPDE. No firm conclusions on the effects of smoking can be drawn from these latter studies because the number of subjects is too small. Uptake of polonium-210 has been examined in bronchial tissues of smokers and nonsmokers; some studies [reviewed in *(38)*] have shown higher concentrations in smokers. Overall, there is considerable evidence that pulmonary carcinogens in cigarette smoke are taken up and metabolized by smokers (as well as by nonsmokers exposed to ETS), but there are still large gaps.

Fewer than 20% of smokers will get lung cancer *(38)*. Susceptibility will depend in part on the balance between carcinogen metabolic activation and detoxification in the smokers. This is an important area requiring intense further study. Most investigations have focused on the activation pathways by quantifying DNA or hemoglobin adducts. Other molecular epidemiologic studies have used genotyping approaches, as discussed later. A series of reports by Bartsch et al. *(123)* provides considerable support for the activation of BaP to form DNA adducts in the lungs of smokers. Earlier investigations [reviewed in *(38)*] demonstrated that cigarette smoke induces aryl hydrocarbon hydroxylase (AHH) activity and proposed a relationship between AHH activity and lung cancer. The AHH assay measures conversion

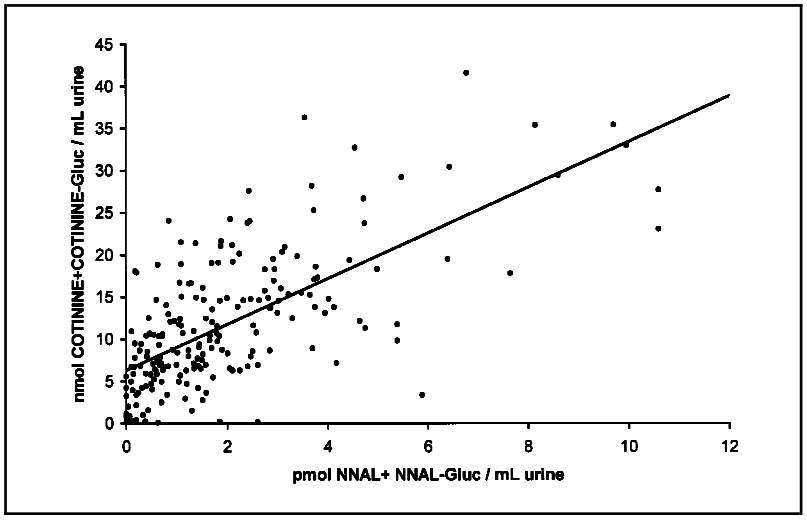

Fig. 5. Correlation between cotinine plus cotinine-*N*-glucuronide (cotinine-Gluc) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) plus its glucuronide (NNAL-Gluc) in smokers' urine (r = .68, two-sided P<.01). Unpublished observations from S. G. Carmella and S. S. Hecht for 223 smokers from whom cotinine and cotinine-*N*-glucuronide were determined by gas chromatography–mass spectrometry, and NNAL and NNAL-Gluc were determined by gas chromatography–nitrosamine selective detection.

of BaP to 3-hydroxy BaP, which is mediated mainly by P4501A1 in human lung *(88–90)*. Therefore, cigarette smoking induces CYP1A1 gene expression. Cancer patients who stopped smoking within 30 days of surgery had elevated levels of AHH activity compared with nonsmoking cancer patients *(124,125)*. Lung tissue from recent smokers with elevated AHH activity also converted BaP-7,8-diol to tetraols to a greater extent than lung tissue from nonsmokers or ex-smokers *(126)*. Subsequently *(127)*, BPDE–DNA adducts were quantified in human lung tissue by high-pressure liquid chromatography–fluorescence of released tetraols; adduct levels were found to be associated with AHH activity in the same samples. In an additional study *(128)*, tetraols released from BPDE–DNA adducts were detected only in lung tissue of individuals null for the glutathione *S*-transferase M1 (GSTM1) gene; among these, a group of subjects with mutated CYP1A1 showed the highest adduct levels. Collectively, these results support the existence of a cigarette smoke-inducible pathway leading to BPDE–DNA adducts in smokers' lungs, as illustrated in Figs. 1 and 4; however, larger studies are necessary to confirm these results. Epidemiologic studies on polymorphisms in carcinogen-metabolizing enzymes, such as CYP1A1 and GSTM1, are discussed in more detail below.

Fluorescence and phosphorescence techniques have also been used to identify tetraols released from BPDE–DNA adducts in human lung *(129,130)*. Many studies *(131–137)* have used immunoassays and ^{32}P-postlabeling to estimate levels of "PAH–DNA adducts" or "hydrophobic DNA adducts" in white blood cells and other human tissues including the lung. Many of these have shown elevated adduct levels in smokers. One series of studies *(138,139)* demonstrated higher levels of adducts and p53 mutations in lung DNA of women than men, consistent with the higher risk for lung cancer observed in some epidemiologic studies *(140)*. However, none of the studies using immunoassays and ^{32}P-postlabeling has identified the structures of the compounds leading to adduct formation. Probably some are PAHs, but individual PAHs differ widely in carcinogenic activity; thus, the results are difficult to interpret. PAH diol epoxides such as BPDE form adducts with hemoglobin and albumin *(141–148)*. Tetraols released by hydrolysis of these adducts have been quantified by gas chromatography–mass spectrometry (GC–MS) and are higher in smokers than in nonsmokers *(146–148)*.

Several studies *(149–154)* have detected 7-methyldeoxyguanosine in human lung. Levels were higher in smokers than in nonsmokers in two of these *(151,154)*, suggesting that NNK may be one source of these adducts. Another likely source is *N*-nitrosodimethylamine. 7-Ethyldeoxyguanosine has also been detected *(149,153)*. While 7-methylguanine is not generally considered as a miscoding adduct, other methyl adducts which do have miscoding properties, such as O^6-methylguanine, are formed at the same time, but at lower levels. One study *(155)* did report the presence of O^6-methylguanine and O^6-ethylguanine in the lung. Pyridyloxobutylated DNA has been detected by GC–MS analysis of lung tissue from smokers in one study, reflecting metabolic activation of NNK or a related nitrosamine, *N′*-nitrosonornicotine; a second study was negative *(153,156)*. The detection of methyl and pyridyloxobutyl adducts in DNA from smokers' lungs is consistent with the ability of human lung tissue to metabolically activate NNK, but the quantitative aspects of the relationship of metabolism to DNA adduct levels are unclear *(22)*. Pyridyloxobutylated globin has also been detected in smokers *(22,157–160)*. Adduct levels in smokers were lower than BPDE globin adduct levels, probably reflecting the lower stability of the diazonium ion intermediates formed from NNK or *N′*-nitrosonornicotine compared with BPDE *(22)*. Levels of 3-ethyladenine in urine were higher in smokers than in nonsmokers; some of the excreted 3-ethyladenine could be formed as a result of metabolic activation of *N*-nitrosodiethylamine *(161,162)*.

DNA repair processes are important in determining whether DNA adducts persist. Because smoking is a chronic habit, one would expect a steady-state DNA adduct level to be achieved by the opposing effects of damage and repair. There are three mechanisms of DNA repair: direct repair, base excision repair, and nucleotide excision repair. These topics have been reviewed elsewhere *(163–165)*. With respect to smoking and lung cancer, direct repair of O^6-methyldeoxyguanosine by O^6-methylguanine–DNA alkyltransferase (AGT) and nucleotide excision repair of PAH–DNA adducts would appear to be the most relevant processes. AGT removes the methyl group from the O^6-position of deoxyguanosine in a stoichiometric reaction, reconverting it to deoxyguanosine *(164)*. Several studies have examined levels of this repair enzyme in tissues from smokers and nonsmokers. In broncho-alveolar lavage cells and peripheral blood mononuclear cells, there was a wide interindividual variation in activity but no effect of smoking *(166)*. In human lung tissue and in human placenta, small but statistically significant increases in AGT activity were observed in smokers compared with nonsmokers *(167,168)*. Expression of AGT was higher in non-small-cell lung carcinomas from smokers than from nonsmokers *(169)*. In smokers, AGT would repair O^6-alkylguanines formed from NNK, *N*-nitrosodimethylamine, or *N*-nitrosodiethylamine. In rats treated with NNK, AGT activity decreases in Clara cells, the nonciliated cells in the epithelial lining of the bronchioles *(170)*. Other studies *(171–173)* show that pyridyloxobutylated DNA inhibits the activity of AGT in mice. BPDE–DNA adducts and other PAH–DNA adducts are repaired by nucleotide excision repair *(174)*. In human cells, repair of BPDE adducts in the hypoxanthine phosphoribosyltransferase gene occurs preferentially in the transcribed strand *(175)*. Repair of the BPDE–DNA adducts is highly dependent on adduct conformation *(176)*. Thus, *cis*-adducts of BPDE with N^2 of deoxyguanosine are repaired more rapidly than *trans*-adducts; rates of repair are also highly dependent on the nature of the base opposite the adduct *(176)*. DNA repair capacity has been studied with respect to lung cancer susceptibility *(177);* this is discussed further below. The effects of smoking on nucleotide excision repair in the human lung do not seem to have been examined.

Free Radicals in Cigarette Smoke and Oxidative DNA Damage

Cigarette smoke contains free radicals and induces oxidative damage in humans. The gas phase of freshly generated cigarette smoke contains up to 600 μg of nitric oxide *(38)*. The particulate phase contains free radicals that are stable enough to be detected by electron spin resonance and spin trapping *(178,179)*. The major free radical species was postulated to be a quinone–hydroquinone complex "held in a tar matrix" *(180)*. Further investigation *(181)* led to the hypothesis that the tar radical system is an equilibrium mixture of semiquinones, hydroquinones, and quinones. It is suggested that this free radical complex causes redox cycling that generates superoxide anion from molecular oxygen and leads to the formation of hydrogen peroxide

and hydroxyl radical *(181)*. The reactive species generated in this cascade cause DNA nicking *(181)*. Other studies *(182–187)* demonstrate that cigarette smoke causes single-strand breaks in DNA of cultured rodent and human cells. Quinone-associated redox cycling may also be involved in these effects; hydroquinone and catechol are believed to play major roles. It has been shown that nitric oxide in the gas phase acts synergistically with cigarette "tar" to cause DNA single-strand breakage in pBR322 plasmid DNA *(188)*. It was suggested that peroxynitrite, generated from nitric oxide and superoxide anion, might be involved in this effect *(188)*. Another study *(189)* also suggests a role for peroxynitrite in oxidative stress induced by aqueous cigarette smoke fractions.

Experiments *in vitro* demonstrate that the gas phase of cigarette smoke causes lipid peroxidation of human blood plasma; this is prevented by the addition of ascorbic acid *(190)*. Both whole-cigarette smoke and gas-phase cigarette smoke cause formation of carbonyls in human plasma *(191)*. Ascorbic acid levels are lower in smokers than in nonsmokers; only smokers consuming more than 200 mg of ascorbic acid per day had serum ascorbate concentrations equivalent to those in nonsmokers who meet the recommended dietary allowance of ascorbic acid *(192)*. Convincing evidence of oxidative damage by cigarette smoke was provided by measurements of increased circulating products of lipid peroxidation (F_2-isoprostanes) in smokers *(193)*. Consistent with these findings and the possible role of reactive oxygen species in DNA damage as discussed above, several studies *(194–196)* have demonstrated moderately increased levels of 8-oxodeoxyguanosine, a miscoding adduct, in DNA from smokers' lungs, leukocytes, and sperm. Increased urinary excretion of 8-hydroxydeoxyguanosine has also been noted *(197,198)*.

Effects of Tobacco Smoke Carcinogens on Tumor Suppressor Genes and Oncogenes

As indicated in Fig. 1, the direct interaction of metabolically activated carcinogens with critical genes, such as the p53 tumor suppressor gene and the Kirsten-ras (KRAS) oncogene, is central to the hypothesis that specific carcinogens form the link between nicotine addiction and lung cancer. In this section, evidence for that link will be considered. By far, the most extensive studies of this type have concerned the p53 tumor suppressor gene. These have been reviewed previously *(34,35)*. The p53 gene plays a central role in the delicate balance of cellular proliferation and death. It is mutated in about half of all cancer types, including more than 50% of lung cancers *(34,35)*. Point mutations at G are common *(34,35)*. In a sample of 550 p53 mutations in lung tumors, 33% were G→T transversions, while 26% were G→A transitions *(36)*. (A purine→pyrimidine or pyrimidine→purine mutation is referred to as a transversion, while a purine→purine or pyrimidine→pyrimidine mutation is called a transition.) A positive relationship between lifetime cigarette consumption and the frequency of p53 mutations and of G→T transversions on the nontranscribed DNA strand also has been noted *(34,35,199)*. These observations are generally consistent with the fact that most activated carcinogens react predominantly at G and that repair of the resulting adducts would be slower on the nontranscribed strand, and thus support the hypothesis outlined in Fig. 1.

However, attempts to link p53 mutations to specific carcinogens or to endogenous processes are more speculative. Many factors will influence the type of mutation. These include the type of DNA adduct formed, the extent to which it is repaired, its sequence context, and the DNA polymerases involved. It is widely stated that G→A transitions at CpG sites in the p53 gene result from deamination of 5-methylC, and although this is a plausible result of endogenous processes that would cause this change, definitive evidence that this occurs *in vivo*, or in the lung, is lacking. Indeed, a recent study *(200)* demonstrates that cytosine methylation greatly enhances guanine alkylation at all CpG sites in the p53 gene by a variety of carcinogens. O^6-Alkylguanines, such as those formed from nitrosamines, are another likely cause of G→A transitions *(28)*. With respect to the origin of G→T transversions in the p53 gene, a study by Denissenko et al. *(201)* is widely quoted as having provided definitive evidence that BPDE is responsible. These investigators did demonstrate that BPDE selectively forms adducts at CpG sites in codons 157, 248, and 273 similar to three major sites of mutation in the p53 gene in lung cancer. However, their studies *(200,202)* also clearly show that methylated CpG sites are targets of a variety of activated carcinogens, as mentioned above. Thus, the results strongly suggest that other DNA-reactive compounds and DNA adducts derived from tobacco smoke would have similar effects. These include diol epoxides of other PAHs, pyridyloxobutylating intermediates derived from NNK and *N′*-nitrosonornicotine, hydroxylamines derived from aromatic amines, as well as acrolein, crotonaldehyde, and 8-oxodeoxyguanosine *(203–208)*. All of these reactive compounds or adducts can cause G→T transversions, although the results may vary depending on other factors such as sequence context as noted above. In summary, while it is likely that cigarette smoke carcinogens are responsible for a substantial percentage of the G mutations observed in the p53 gene from human lung cancers, the assignment of these mutations to specific carcinogens is at best speculative.

Mutations in codon 12 of the KRAS gene are found in 24%–50% of human primary adenocarcinomas but are rarely seen in other lung tumor types *(31–33)*. These mutations are more common in smokers and exsmokers than in nonsmokers, which suggests that they may be induced by direct reaction with the gene of an activated tobacco smoke carcinogen *(33)*. The most commonly observed mutation is GGT→TGT, which typically accounts for about 60% of the codon 12 mutations, followed by GGT→GAT (20%) and GGT→GTT (15%). This is quite similar to the pattern observed in lung tumors from mice treated with BaP, 5-methylchrysene, and benzo[*b*]fluoranthene, three pulmonary carcinogens found in tobacco smoke *(209)*. However, caution is again required because numerous constituents of tobacco smoke, as discussed above, can induce G→T transversions. Moreover, whereas Kras mutations are commonly observed in mouse lung tumors, they are rarely found in rat lung tumors, such as those induced by NNK; rodent lung tumors also rarely contain mutated p53 genes *(22,210)*. In the mouse, the O^6-methylguanine pathway of NNK metabolic activation is dominant, resulting in a high percentage of GGT→GAT mutations in codon 12 of Kras *(22)*. But pyridyloxobutylation leads to more G→T than G→A mutations in codon 12 *(207)*. In the rat, both pyridyloxobutylation and methylation pathways are critical in lung tumorigenesis by NNK *(22)*. We do not know the relative importance of these pathways in human lung. If pyridyloxobutylation is critical, as in the rat, a higher percentage of G→T transversions would be expected as a result of NNK exposure than is observed in mice.

The $p16^{INK4a}$ tumor suppressor gene is inactivated in more than 70% of human non-small-cell lung cancers, via homozygous deletion or in association with aberrant hypermethylation of the promoter region *(211–213)*. In the rat, 94% of adenocarcinomas induced by NNK were hypermethylated at the p16 gene promoter *(213)*. This change was frequently detected in hyperplastic lesions and adenomas, which are precursors to the adenocarcinomas induced by NNK. Similar results were found in human squamous cell carcinomas of the lung *(213)*. The p16 gene was coordinately methylated in 75% of carcinoma *in situ* lesions adjacent to squamous cell carcinomas that had this change. Methylation of p16 was associated with loss of expression in tumors and precursor lesions, indicating functional inactivation of both alleles. Aberrant methylation of p16 has been suggested as an early marker for lung cancer *(213)*. The expression of cell cycle proteins is related to the p16 and retinoblastoma (RB) genes; NNK-induced mouse lung tumors appear to resemble human non-small-cell lung cancer in the expression of cell cycle proteins *(214)*. The estrogen receptor gene is also inactivated through promoter methylation. There was concordance between the incidence of promoter methylation in this gene in lung tumors from smokers and from NNK-treated rodents *(215)*.

Loss of heterozygosity and exon deletions within the fragile histidine triad (FHIT) gene are associated with smoking habits in lung cancer patients and have been proposed as a target for tobacco smoke carcinogens *(216)*. However, point mutations within the coding region of the FHIT gene were not found in primary lung tumors. Data are insufficient at present to attempt to relate these changes to specific carcinogens.

Molecular Epidemiologic Studies Involving Possible Gene–Carcinogen Interactions and Related Factors

Molecular epidemiology attempts to integrate biomarkers into epidemiologic investigations, thus providing mechanistic insights into cancer susceptibility with the ultimate goal of identifying individuals at high risk. This has been the subject of recent reviews *(217–220)*. Lung cancer, in particular, has been studied quite extensively with respect to potential interactions between carcinogen metabolizing enzymes and tobacco smoke carcinogens, with the aim of identifying smokers at high risk for this disease. These studies *(218–220)* have been reviewed by a number of authors. Among genes for carcinogen-metabolizing enzymes, polymorphisms [variants occurring in more than 1% of the population *(217)*] in the cytochrome P450 genes CYP1A1, CYP2D6, CYP2E1 and in mu-class glutathione *S*-transferase (GSTM1) have received the most attention.

The CYP1A1 gene product, P4501A1 or AHH, is inducible by cigarette smoke in human lung and is involved in the metabolism of PAHs. Polymorphisms in this gene and their relationship to lung cancer risk have been discussed elsewhere *(221–224)*. While there is some evidence that a CYP1A1 polymorphism may confer higher lung cancer risk in Japanese people, this has not been generalizable to other populations *(220,221)*. Limited data are available on the functional significance of such polymorphisms with respect to PAH metabolism and carcinogenesis *(222–224)*. There is no doubt that P4501A1 is important in the metabolism of PAHs, as illustrated for BaP in Fig. 4. However, P4501A1 is involved both in the metabolic activation of BaP to BPDE and in its detoxification (via formation of 3-hydroxy BaP and other metabolites). Therefore, it is not clear what the meaning of a variant CYP1A1 genotype would be with respect to lung cancer risk.

The CYP2D6 gene product metabolizes drugs such as the antihypertensive drug debrisoquine. The role of this gene as a risk factor for lung cancer has been extensively studied since the original report demonstrating under-representation of the poor-metabolizing phenotype in smokers diagnosed with lung cancer *(220,225–227)*. Numerous subsequent studies have examined this relationship using various approaches; the literature has been reviewed elsewhere *(220,226)*. P450 2D6 is expressed primarily in the liver; enzyme activity, protein expression, and gene expression have not been reported in human lung *(220)*. A recent case–control study *(227)* identified inactivating mutations at the CYP2D6 locus as well as mutations that impair but do not abolish enzyme activity. Compared with subjects with homozygous-inactivating mutations, no association with lung cancer was observed for those individuals with homozygous or heterozygous functional alleles *(227)*. Overall, evidence for a role of CYP2D6 polymorphisms as a risk factor for lung cancer is weak, conflicting, and inconclusive *(220,226,227)*. On the basis of two studies that showed that P4502D6 can activate NNK *(228,229)*, this carcinogen is frequently mentioned as the substrate for P4502D6 that would be relevant to the proposed lung cancer risk. However, other studies [reviewed in *(22)*] definitively show that P4502D6 is at most a minor contributor to NNK metabolism. Therefore, there is little theoretical basis for a role of CYP2D6 in lung cancer, which is consistent with the inconclusive molecular epidemiologic studies discussed above.

The CYP2E1 gene product is involved in the metabolism of low-molecular-weight compounds, such as ethanol, 1,3-butadiene, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, and, to a minor extent, NNK. It is expressed in human liver, kidney, brain, and lung *(220)*. The possible association of CYP2E1 polymorphisms with lung cancer has been reviewed elsewhere *(220)*. The evidence up until that time was generally inconclusive, but a recent study *(230)* showed a 10-fold decrease in risk for overall lung cancer and adenocarcinoma with variant genotypes of CYP2E1, consistent with a role for *N*-nitrosamines in adenocarcinoma of the lung.

The GSTM1 gene codes for M or mu class glutathione *S*-transferases that are involved in the detoxification of various carcinogens including PAH diol epoxides. Other human glutathione *S*-transferases fall into the alpha, pi, and theta classes, and these are named GSTA, GSTP, or GSTT, respectively *(76)*. Approximately 40%–50% of the human population has the GSTM1 null genotype *(220)*. A large number of studies have examined the relationship between GSTM1 null and lung cancer risk, testing the hypothesis that risk would be elevated in GSTM1 null individuals. These studies have been reviewed elsewhere *(220)*. Collectively, the data suggest that there may be a modest association of GSTM1 null with lung cancer *(220,231,232)*. A recent study *(233)* indicates a higher risk for lung cancer in females than in males with GSTM1 null. GSTP1-1 and GSTA1-1 also are important catalysts of glutathione conjugation of BPDE (Fig. 4) and other PAH diol epoxides *(87)*. Moreover, the content of GSTP1 in the human lung significantly exceeds that of GSTM1 *(85)*. These facts indicate that detoxification of PAH diol epoxides is a complex process, which is unlikely to be controlled by the absence or presence of a single gene product.

In summary, the hypothesis that lung cancer risk depends in part on carcinogen activation and detoxification is attractive, but it cannot be adequately tested by single genotyping approaches. Carcinogen metabolism is simply too complex (Fig. 4). Some studies *(220,234)* have also examined multiple genotypes, and several have found increased risk associated with variants of CYP1A1 in combination with GSTM1 null genotype. Further studies of this type are required and will be aided by the emerging DNA microarray technology, which will allow rapid, multiple genotyping. Carcinogen metabolite phenotyping, which would give a composite view of activation and detoxification reactions in humans, is likely to be an even more useful approach, although potentially more technically demanding than genotyping.

Another approach for assessing individual susceptibility to carcinogenic agents is the mutagen sensitivity assay, in which the frequency of *in vitro* bleomycin-induced chromatid breaks is quantified [reviewed in *(177,235)*]. In a case–control study, mutagen sensitivity was significantly associated with lung cancer risk. This methodology has been extended, using BPDE for induction of chromosomal aberrations in human lymphocytes. Mutagen sensitivity was found to be greater in lung cancer case patients than in control subjects *(177)*. In other studies [reviewed in *(177)*], reduced DNA repair capacity was associated with increased lung cancer risk. The results of these studies are generally consistent with the scheme shown in Fig. 1, although they lack specificity with respect to the particular carcinogens or enzymes involved.

Evaluation of the Roles of Specific Carcinogens in Human Lung Cancer Induced by Cigarette Smoke

Table 3 summarizes the data discussed above and estimates the role of various groups of carcinogens found in cigarette smoke as contributors to human lung cancer. The criteria used for evaluation are the presence of the compounds in cigarette smoke; their pulmonary carcinogenicity in laboratory animals; their human uptake, metabolism, and adduct formation; and their possible role in causing molecular changes in oncogenes or suppressor genes. The evaluation focuses on data for specific compounds and uses a weight-of-the-evidence approach.

The strongest evidence is for PAHs and NNK. There is no question that these compounds are present in mainstream and sidestream smoke of both nonfilter and filter cigarettes; extensive studies on their concentrations have been reported *(38,65–67)*. There is also no doubt that NNK and some PAHs are very effective pulmonary carcinogens in rodents. Their uptake by smokers has been clearly demonstrated. Human liver metabolizes PAHs and NNK; one study *(100)* demonstrated that the relative rates of oxidative metabolism to electrophiles or their precursors were NNK>BaP>NNAL. Human lung metabolically activates BaP, in part by P4501A1, which is induced by cigarette smoking. Human lung converts NNK to NNAL; however, the activation of NNK in this tissue occurs less extensively than in rodent lung *(22)*. Human bronchial epithelial cells are transformed by NNK *(236)*. BPDE–DNA adducts have been detected in human lung, as have methyl and pyridyloxobutyl adducts, but these adduct studies are limited to date. Mutations in the p53 and KRAS genes could be caused by activated metabolites of PAHs, NNK, and many other carcinogens. Collectively, these data provide strong evidence for a role of PAHs and NNK as causes of lung cancer in smokers according to the overall mechanism outlined in Fig. 1, although there are some important gaps. The relative roles of BaP and NNK have been discussed previously *(22)*. On the basis of decreases in concentrations of BaP and increases in levels of NNK in cigarette smoke as well as on biologic and pharmacokinetic considerations, it is plausible that NNK is partially responsible for the dramatic increase in adenocarcinoma of the lung, which has now surpassed squamous cell carcinoma as the leading type of lung cancer in the United States; other factors, such as changes in puff volume and genetic influences, have been discussed elsewhere *(22,230,237,238)*.

Studies on aza-arenes are quite limited. Two aza-arene pulmonary carcinogens listed in Table 1, B, occur in extremely small quantities in cigarette smoke, and nothing is known about their disposition and metabolism in human systems. Metals are clearly present in cigarette smoke, and some are potent pulmonary carcinogens. But the role of these compounds is clouded by our limited knowledge of their valence state in cigarette smoke or after inhalation. Among miscellaneous organic compounds, the concentrations of 1,3-butadiene and aldehydes, such as formaldehyde and acetaldehyde, in cigarette smoke are substantial *(38)*, but their role as pulmonary carcinogens is not clear. There is little doubt that cigarette smoke can cause oxidative damage,

Table 3. Evaluation of roles of specific carcinogens in human lung cancer induced by cigarette smoke

Compound(s)	Evaluation of evidence for a role in lung cancer*					
	Presence in cigarette smoke	Pulmonary carcinogenicity in rodents	Human uptake	Human metabolism and adduct formation	Molecular changes in human genes	Overall score
Specific PAHs†	4	4	4	3	3	18
Aza-arenes	3	3	1	1	2	10
NNK,‡ *N*-nitrosodiethylamine	4	4	4	3	3	18
Metals§	4	4	1	1	1	11
Miscellaneous organic compounds‖	4	3	1	1	1	10
Free radicals/oxidative damage	3	1	3	3	1	11

*Scores: 1 = Inadequate data; 2 = weak or equivocal evidence; 3 = some evidence; limited studies; and 4 = clear evidence; strong, reproducible studies.

†Polycyclic aromatic hydrocarbons (PAHs) including benzo[*a*]pyrene, benzofluoranthenes, dibenzo[*a,i*]pyrene, dibenz[*a,h*]anthracene, and 5-methylchrysene. Does not include studies of "PAH–DNA adducts" or "hydrophobic DNA adducts" as determined by immunoassay and ^{32}P-postlabeling (*see* text).

‡4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone.

§Nickel, chromium, cadmium, polonium-210, and arsenic.

‖Including 1,3-butadiene, ethyl carbamate, and aldehydes.

and it contains substantial quantities of free radicals such as nitric oxide; other radical species seem to be present but are poorly characterized. There is presently no evidence that the oxidative damage caused by cigarette smoke is specifically implicated in lung cancer. The lack of a protective effect against lung cancer of β-carotene, an antioxidant, in three human chemoprevention trials *(239–241)* and the lack of a protective effect of α-tocophenol in one of them *(241)* may indicate that oxidative damage is unimportant as a cause of cigarette smoke-induced lung cancer, although other explanations for the failure of these trials are plausible.

CONCLUSIONS

The complexity of tobacco smoke causes confusion in the literature about the mechanisms by which it induces lung cancer. Some authors oversimplify by referring to this complex mixture as "tar" or by attempting to implicate only one substance—such as BPDE—in cancer causation, while others maintain that the level of complexity is such that the mechanism is unknown. The reality lies between these extremes. A theme of this review is that mechanistic insight can be gained by focusing on specific pulmonary carcinogens in cigarette smoke.

Cigarette smoke carcinogens form the link between nicotine addiction and lung cancer (Fig. 1). Collectively, the evidence favoring the sequence of steps illustrated in Fig. 1 is extremely strong, although there are important aspects of each step that require further study. These include carcinogen metabolism and DNA binding in human lung, the effects of cigarette smoke on DNA repair and adduct persistence, the relationship between specific carcinogens and mutations in critical genes, and the sequence of gene changes leading to lung cancer.

Although there are at least 55 carcinogens in cigarette smoke (Table 1, A), presently available data allow us to focus on 20 substances that are probably involved in lung cancer induction because of their presence in cigarette smoke and their pulmonary carcinogenicity in laboratory animals (Table 1, B). By use of a weight-of-the-evidence approach, specific PAHs and the tobacco-specific nitrosamine NNK can be identified as probable causes of lung cancer in smokers, but the contribution of other agents cannot be excluded (Table 3). The long-term exposure of smokers to the genotoxic intermediates formed from these carcinogens is consistent with our present understanding of cancer induction as a process which requires multiple genetic changes. Thus, it is completely plausible that the continual barrage of DNA damage produced by tobacco smoke carcinogens causes the multiple genetic changes that are associated with lung cancer. While each dose of carcinogen from a cigarette is extremely small, the cumulative damage produced in years of smoking will be substantial.

Aspects of the scheme illustrated in Fig. 1 are well understood for PAHs and NNK. A great deal is known about the metabolic activation and detoxification of these compounds, although there are still parts of these complex pathways (Fig. 4) that require clarification. There is a good general understanding of the mechanisms by which these tobacco smoke carcinogens interact with DNA to form adducts, and considerable information is available about the repair, persistence, and miscoding properties of these adducts. There are many aspects of these processes that require further study, however. In particular, little is known about the levels, persistence, and repair of specific carcinogen DNA adducts in the lungs of smokers or the effects of chronic smoking on these factors. The location of carcinogen adducts at specific sites in human DNA has not been studied, mainly because of limitations in sensitivity. Nevertheless, one can reasonably conclude that metabolically activated tobacco smoke carcinogens directly cause mutations observed in tumor suppressor genes and oncogenes, although details remain elusive since numerous DNA-damaging agents in tobacco smoke cause similar mutations.

Many molecular epidemiologic studies attempting to identify gene–carcinogen interactions and other mechanistic aspects of the lung cancer process have focused on smokers. It is very important to elucidate those factors that determine which smokers will be susceptible to lung cancer development and to find natural protective mechanisms. Although the results to date are of great interest, these studies have not yet reached their full potential. Most have focused on individual genotypes which may be expected to affect particular reactions involved in metabolic activation or detoxification. Some of this research has been driven by the availability of relatively simple genotyping techniques. As this field evolves, it is becoming increasingly clear that this approach will yield only limited information. A more comprehensive integration of genotype and phenotype biomarkers into epidemiologic studies is required. This will be enhanced by the rapidly developing DNA microarray technology (DNA chips) that will allow rapid multiple genotyping. Ultimately, it should also be possible to monitor the metabolic pathways illustrated in Fig. 1 in smokers and in other people exposed to tobacco carcinogens by a combined genotyping–phenotyping approach. This would lead to methods for identification of susceptible individuals and early detection of lung cancer.

Blocking any of the horizontal pathways in Fig. 1 should lead to reduced lung cancer incidence and mortality. Preventing nicotine addiction and improving smoking cessation strategies are clearly priorities, but these are only partially successful *(242–244)*. An important approach for addicted smokers and ex-smokers is chemoprevention. Many agents that can block carcinogen activation or enhance detoxification are now known *(245)*. Other chemopreventive compounds inhibit events downstream from DNA adduct formation *(246–249)*. The further development of effective chemopreventive agents should be a major priority for reducing lung cancer incidence.

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NOTES

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