Approaches to Cancer Prevention Based on an Understanding of *N*-Nitrosamine Carcinogenesis (44168)

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Abstract. **Understanding carcinogenesis is critical for development of rational approaches to cancer prevention. This paper uses *N*-nitrosamine carcinogenesis as an example. *N*-Nitrosamines are a large group of potent carcinogens. Approximately 300 different *N*-nitrosamines are carcinogenic. At least 30 animal species are responsive to their effects. There is little doubt that humans exposed to sufficient amounts of *N*-nitrosamines would also be susceptible to their carcinogenic effects. Human exposure to preformed *N*-nitrosamines occurs through the diet, in certain occupational settings, and through the use of tobacco products, cosmetics, pharmaceutical products, and agricultural chemicals. Diminishing human exposure to these carcinogens is one approach to prevention of cancer, and this has been accomplished in many instances, although exposure to *N*-nitrosamines in tobacco products is still unacceptably high. Human exposure to *N*-nitrosamines also occurs by nitrosation of amines in the body, *via* their acid or bacterial catalyzed reaction with nitrite, or by reaction with products of nitric oxide generated during inflammation or infection. A second approach toward prevention of *N*-nitrosamine carcinogenesis is inhibition of this endogenous *N*-nitrosamine formation. Substantial reductions have been achieved with ascorbic acid and other nitrite scavengers. *N*-Nitrosamines undergo a simple cytochrome P450-mediated metabolic activation step, which is critical for their carcinogenicity. The third approach involves the use of chemopreventive agents that block this step, or other steps in the carcinogenic process. A large number of potent chemopreventive agents against nitrosamine carcinogenesis have been identified. Chemoprevention of lung cancer induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is discussed as an example of this approach.** [P.S.E.B.M. 1997, Vol 216]

Understanding carcinogenesis is the key to developing rational approaches to cancer prevention. In this paper, I will use *N*-nitrosamines as an example, but the general strategy could be applied to any class of carcinogen. *N*-Nitrosamines, referred to as nitrosamines in this paper, are one of the most extensively studied groups of carcinogens. A large number of nitrosamines are potent carcinogens in a wide variety of animal species. Nitrosamines occur in the human environment and are formed endogenously in humans. They must undergo metabolism to exert their carcinogenic effects. The metabolic activation reactions that lead to DNA damage and cancer are well understood in many cases. There are three major strategies that are being used to prevent cancer induction by nitrosamines: prevention of exposure to preformed nitrosamines, prevention of endogenous nitrosamine formation, and chemoprevention of nitrosamine carcinogenesis. Examples of these strategies will be given within the context of an overall discussion of nitrosamine carcinogenesis.

Carcinogenicity of Nitrosamines

In 1954, Barnes and Magee reported that *N*-nitrosodimethylamine (NDMA), the simplest dialkylnitrosamine, was a potent toxin in the rat, mouse, rabbit, and dog causing severe hemorrhagic centrilobular necrosis of the liver (1). This report was followed by the seminal observation by Magee and Barnes in 1956 that prolonged feeding of a diet containing 50 ppm of NDMA caused malignant hepatic neoplasia in rats (2). In 1937, Freund had reported fatal

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0037-9727/97/2162-0181$10.50/0

human exposure of research chemists to NDMA (3). More recently, homicidal NDMA poisoning was reported in the United States and Germany (4). Acute poisoning resulted in massive hepatic necrosis. Analysis of the hepatic DNA of the American victims demonstrated the presence of adducts resulting from the metabolic activation of NDMA (5).

The initial report of NDMA carcinogenesis spurred further research into the carcinogenic properties of other nitrosamines by Druckrey, Preussmann, Schmähl, Lijinsky, and others (6–10). These tests were facilitated by the ready availability of nitrosamines, which can be synthesized by simple nitrosation of the corresponding amines. The carcinogenic properties of nitrosamines have been extensively documented and reviewed (6–8). Most bioassays have been carried out in rats, hamsters, and mice. Approximately 300 nitrosamines have been shown to be carcinogenic (6–10). Over 30 different species are responsive to nitrosamine carcinogenesis (10). These data, together with biochemical data indicating similar metabolic pathways in laboratory animals and humans, strongly indicate that nitrosamines are also carcinogenic in humans (7, 8).

Several points are noteworthy with respect to nitrosamine carcinogenesis. First, a variety of target organs are affected, and the target selectivity depends on nitrosamine structure and often on the species employed. Tumors of the liver, esophagus, lung, nasal mucosa, bladder, tongue, and forestomach are commonly induced by nitrosamines in rats (6–8). Different target organs are frequently affected in hamsters. The most notable difference between rats and hamsters is the esophagus, which is the most common target tissue in the rat but is not affected in the hamster (8). In contrast, the hamster pancreas is more sensitive to nitrosamine carcinogenesis than that of the rat. Nitrosamines are often the compounds of choice for the induction and study of specific types of tumors in laboratory animals (6–8). For example, *N*-nitrosomethylbenzylamine (NMBA) is widely employed to study esophageal cancer as it readily induces these tumors in rats. *N*-nitroso*bis*-2-(oxopropyl)amine (BOP) is used for induction of pancreatic tumors in Syrian golden hamsters, *N*-nitrosobutyl(4-hydroxybutyl)amine (BHBN) reproducibly causes bladder tumors in rats, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is extensively used for induction of lung tumors in rats and mice.

A second important point is that nitrosamines frequently induce tumors at specific sites independent of the route of administration (6–8). Good examples are NMBA for the rat esophagus and NNK for the rat lung.

Third, some nitrosamines are extremely powerful carcinogens, inducing tumors at very low doses. For example, dose-response studies, using 4080 rats, were carried out on NDMA and *N*-nitrosodiethylamine (NDEA) administered in the drinking water (9). At doses sufficiently high for the median time to death to be estimated, the following equation was derived: Dose Rate $\times$ Median^n = Constant, where n was about 2.3, or 1, depending on the tumor type. At doses sufficiently low for longevity to be nearly normal (about 2.5 years), liver tumor incidence was simply proportional to dose rate. There was a linear relationship observed at doses below 1 ppm. Thus, a dose of 1 ppm caused about 25% of the rats to develop a liver neoplasm, 0.1 ppm about 2.5%, etc., with no indication of a threshold.

Prevention of Human Exposure to Preformed Nitrosamines

The relative ease of nitrosamine formation from commonly occurring secondary and tertiary amines suggested that there would be numerous situations in which human exposure to these potent carcinogens could occur. The development of reliable analytical methods for nitrosamine detection was essential for delineation of nitrosamine exposure. A major advance was the development of a nitrosamine selective detector, the Thermal Energy Analyzer (11). The N-N = O bond is thermally cleaved, and the released nitrosyl radical is oxidized by ozone to excited state NO_2, which returns to the ground state with emission of light in the near infrared region of the spectrum. The emitted light is monitored by an infrared sensitive photomultiplier tube. Coupling of this detector to a gas chromatograph provides a very sensitive and reliable method for the detection and quantitation of nitrosamines, provided that they have sufficient volatility. This method has been widely applied.

Exogenous exposure to preformed nitrosamines can occur in the diet, in certain occupational settings, and through use of tobacco products, cosmetics, pharmaceutical products, and agricultural chemicals (12–14). In several cases, significant reductions of human exposure to preformed nitrosamines have been achieved.

Dietary Exposure. The use of nitrite as a preservative in cured or smoked meat or fish products raised concerns about nitrosamine formation, since the reaction of nitrite with amines occurs readily under a variety of conditions. Nitrite inhibits formation of a toxin by the anaerobic spore-forming bacteria *Clostridium botulinum*. Nitrite is also responsible for the pink color associated with nitrite cured meats and stabilizes the flavor of stored meats by preventing undesirable oxidation products (15). Before the introduction of process modifications and the use of ascorbate as an inhibitor, levels of nitrosamines in products such as fried bacon were up to 100 ppb, but this has decreased substantially. Nitrosamines are now generally found in concentrations of <10 ppb in cured meats; the main nitrosamines detected are NDMA and NPYR (15). Other foods and beverages that contain detectable levels of these nitrosamines, usually <10 ppb, include cheese, beer, and certain milk products. Process modifications have resulted in important reductions of the levels of nitrosamines in beer (from 5–20 ppb to less than 0.4 ppb). As a result of these modifications, the average daily intake of NDMA and NPYR through the diet is of the order of 1 μg/person in many industialized countries (13). The reductions in dietary

nitrosamine exposure are good examples of successful cancer prevention strategies.

Occupational Exposure. Occupational exposure to nitrosamines occurs in the rubber, leather, and metal industries (12, 13, 16, 17). NDMA, NDEA, *N*-nitrosomorpholine (NMOR), and *N*-nitrosopiperidine have been detected in the air of rubber factories. Exposure levels have been substantially reduced in Germany, from a maximum of 380 $\mu g/m^3$ to 41 $\mu g/m^3$. New regulations on occupational exposure limits in Germany require levels not greater than 2.5 $\mu g/m^3$ (17). Accelerators and retarders employed in the vulcanization process are the sources of the nitrosamines. Workers in the rubber industry are at an increased risk for cancer but it is uncertain whether this is due to nitrosamine exposure. The reductions that have been achieved are significant, but there is still room for further improvement.

In the leather tanning industry, the highest concentration of NDMA reported was 47 $\mu g/m^3$; following cleaning of the factory, NDMA concentration decreased to 0.1–3.4 $\mu g/m^3$ (12). The presence of NDMA has been attributed to the use of dimethylamine sulphate in the hair removal process. Dimethylamine can be released and react with nitrogen oxides in the factory air.

In the metal industry, substantial quantities of *N*-nitrosodiethanolamine (NDELA), up to 2%, have been detected in cutting fluids (12, 13). NDELA is formed by the reaction of nitrite, a corrosion inhibitor, with diethanolamine or triethanolamine. NDELA levels in cutting fluids have been dramatically reduced in Germany, but may still reach high levels in other industrialized countries.

Tobacco Products. Tobacco products are the greatest source of nonoccupational exposure to nitrosamines (12, 13, 18–22). In commercial cigarettes, volatile nitrosamines such as NDMA and NPYR typically occur in the range of 2–20 ng/cigarette. Levels of these compounds in cigarette sidestream smoke, the main component of environmental tobacco smoke, are up to 100 times greater than in mainstream smoke. "Tobacco-specific nitrosamines" are also present in tobacco products, and their levels are much greater than those of the volatile nitrosamines. Tobacco-specific nitrosamines are formed during the curing and processing of tobacco by nitrosation of nicotine and related alkaloids of tobacco. The structures of the seven tobacco-specific nitrosamines that have been identified in tobacco products are illustrated in Figure 1. NNK, NNN, *N'*-nitrosoanabasine (NAB), and *N'*-nitrosoanatabine (NAT) have all been identified in cigarette smoke and their levels well characterized. Typical levels per cigarette, in ng, are: NNK, 100; NNN, 220; NAB, 20; and NAT, 160. Among these compounds, NNK and NNN are the strongest carcinogens and are believed to play an important role in tobacco-induced cancer. Levels of tobacco-specific nitrosamines in cigarette smoke are influenced by many factors including levels in tobacco, nitrate concentration in tobacco, tobacco type, and smoking parameters (22). NNK concentrations in a typical American nonfilter cigarette have increased over the past 20 years, probably due to increased nitrate (23).

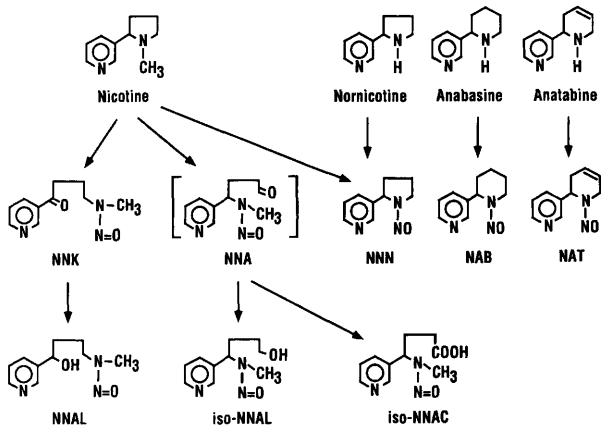

Figure 1. Structures of tobacco-specific nitrosamines and their alkaloid precursors. NNK, NNN, NAB, NAT, NNAL, iso-NNAL, and iso-NNAC have been detected in tobacco products.

Levels of tobacco-specific nitrosamines are remarkably high in unburned tobacco, particularly snuff that is consumed orally in a practice called snuff-dipping. In addition to NNK, NNN, NAB, and NAT, unburned tobacco also contains NNAL, iso-NNAL, and iso-NNAC. Moreover, snuff products contain substantial amounts of nitrosamino acids. In total, 23 different nitrosamines have been identified in processed tobacco. Typical levels of tobacco-specific nitrosamines in snuff, in $\mu g/g$, are: NNK, 3.6; NNN, 17.5; NAT, 15.6; and iso-NNAC, 1.1. Process modifications and storage strongly influence levels of tobacco-specific nitrosamines in snuff. Levels of these carcinogens have been reduced in some snuff products, but they are still much higher than in any other consumer product (22).

Cosmetics. Nitrosamines were first reported in cosmetics products in 1977 (12, 24). More than 8000 raw materials are used in formulating cosmetic products and many of these are amines and related compounds. The raw materials function as emulsifiers, thickeners, etc. Sometimes they are contaminated with nitrosatable amines. Nitrite is sometimes present as a contaminant, and some preservatives used in cosmetics products can release nitrite. *N*-Nitrosodiethanolamine (NDELA) is the most commonly detected nitrosamine in cosmetics. It is formed by nitrosation of triethanolamine or diethanolamine. In the United States, its levels have decreased somewhat, but in 1992 there was still up to 3000 ppb in some products. NDELA has substantial hepatocarcinogenicity in the rat (8). Other nitrosamines detected in cosmetics include *N*-nitrosomethyl-*N*-dodecylamine, *N*-nitrosomethyl-*N*-tetradecylamine, and *N*-nitrosomethyl-*N*-octadecylamine. Sunscreens have been found to contain up to 21 ppm of 2-ethylhexyl 4-(*N*-methyl-*N*-nitrosamino)-benzoate.

Pharmaceutical and Agricultural Products. Many drugs that are secondary or tertiary amines can potentially be nitrosated (12–14). The greatest risk for nitrosamine formation from pharmaceutical products is through

endogenous nitrosation in the stomach. Administration of drugs and nitrite to laboratory animals has resulted in tumor induction, presumably by nitrosamine formation (14, 25). The analgesic drug aminopyrine, which has been banned in Germany, is rapidly nitrosated to give NDMA *in vivo;* it also contained substantial levels of this carcinogen. Generally, however, nitrosamine contamination of drugs is rare (13). Several routes can lead to nitrosamine contamination in pesticides (12). These include the use of contaminated starting materials for synthesis, various side reactions, and the use of nitrite as a corrosion inhibitor for metal containers. Nitrosamine contamination has been limited mainly to dinitroaniline herbicides, dimethylamine salts of phenoxyalkanoic acid herbicides, diethanolamine and triethanolamine salts of acid pesticides, quaternary ammonium compounds, and morpholine derivatives. In general, the main exposure to nitrosamines in these products is confined to workers employed in their preparation and to farmers using contaminated products (12).

Summary of Exogenous Exposures to Nitrosamines. In nonoccupational settings, tobacco products represent by far the greatest source of nitrosamine exposure, in spite of the vast amount that is known about nitrosamine formation in these products. Total exposure to volatile and tobacco-specific nitrosamines is at least 10 times greater through inhalation of cigarette smoke than by dietary exposure or by contact with other products. A person who smokes 20 cigarettes/day will inhale about 10 μg of carcinogenic nitrosamines, while dietary exposure seldom exceeds 1 μg/day. Exposure to nitrosamines through snuff-dipping is likely to be about 10 times greater than from cigarette smoking. The high exposure to carcinogenic nitrosamines through use of tobacco products contrasts with the significant reductions that have been achieved in other consumer products and represents a major failure of regulatory mechanisms.

Occupational exposures to nitrosamines may still be significant, especially in the rubber and metal-working industries. Improved control of nitrosamine exposure is required in occupational settings.

Endogenous Formation of Nitrosamines and Its Prevention

Endogenous exposure to nitrosamines occurs by nitrosation of amines in the body, *via* their acid or bacterial catalyzed reaction with nitrite, or by reaction with products of nitric oxide generated during inflammation and infection.

Nitrosamines can readily form in the acidic environment of the stomach (14, 25). Under acidic conditions, nitrite will form nitrous acid (HNO_2), which dimerizes with loss of water to give N_2O_3. This reacts with amines producing nitrosamines. Thus, animals exposed to amines and nitrite will develop tumors typical of the resulting nitrosamines. However, nitrosamine formation is not limited to the acidic environment of the stomach. Activated macrophages and other cell types produce NO from arginine by the inducible NO synthase pathway (13, 14, 26–28). Thus, under conditions of chronic inflammation or infection, substantial amounts of NO are produced. This reacts with dissolved oxygen to give N_2O_3 and N_2O_4, which can nitrosate amines. Nitrosamine production has been confirmed in animals treated with lipopolysaccharide or infected with hepatitis virus (27, 28). Bacterial strains isolated from human infections can also catalyze nitrosation of amines (13).

The endogenous formation of nitrosamines in humans has been repeatedly demonstrated by analysis of nitrosamino acids, particularly *N*-nitrosoproline (NPRO), in urine (29). Human urine contains several nitrosamino acids: NPRO, *N*-nitrososarcosine (NSAR); *N*-nitrosothiazolidine-4-carboxylic acid (NTCA); *trans*- and *cis*-isomers of *N*-nitroso-2-methylthiazolidine 4-carboxylic acid (NMTCA); 3-(*N*-nitroso-*N*-methylamino)propionic acid (NMPA); *N*-nitrosotetrahydro-4*H*-1,3-thiazine 4-carboxylic acid (NTHTCA); and *N*-nitrosoazetidine-2-carboxylic acid (NAZCA). The most prevalent are NPRO, which is normally excreted in amounts of about 3 μg/day, and NTCA, NMTCA, and NSAR, total about 25 μg/day. Since NPRO is noncarcinogenic and is not metabolized, studies on its formation have been carried out in humans who ingested nitrate and proline with or without dietary nitrosation modifiers. The results of these studies demonstrated that *in vivo* nitrosation occurs in humans and can be inhibited by dietary constituents such as vitamins C and E. A test for endogenous nitrosation—the NPRO test—has been employed widely in studies of human populations (13, 14, 26). Using this test, elevated exposure to nitroso compounds has been demonstrated in subjects at high risk of various cancers including stomach, esophagus, oral cavity, nasopharynx, and aerodigestive tract. Endogenous nitrosation of proline also occurs in smokers. Endogenous nitrosation can be inhibited by ascorbic acid, which acts as a nitrite scavenger. Results from several studies in which moderate doses of ascorbic acid were taken after each meal are summarized in Table I (13). These studies showed that ascorbic acid lowered the body burden of intragastrically formed nitroso compounds in subjects living in high-risk areas for cancers of various types. The reductions in endogenous nitrosation observed in these studies are consistent with the epidemiologic evidence indicating that vegetables and fruits are protective against cancer (30). Vegetables and fruits are a good source of vitamins C and E, as well as polyphenols, and these are all good nitrosation inhibitors in humans.

Metabolic Activation and Detoxification of Nitrosamines

Nitrosamines are relatively unreactive and require enzymatic activation to intermediates, which bind to DNA, initiating the carcinogenic process (31). There are competing detoxification reactions. The metabolic activation of nitrosamines is catalyzed by members of the cytochrome P450 enzyme family. Hydroxylation of the carbon atom next to the nitrosamino group, a reaction called α-

Table I. Inhibition of Endogenous Nitrosation by Ascorbic Acid (po 200–500 mg/Day/Person, after Meals) in Subjects at High Risk for Various Cancers

Cancer site/study subjects	% inhibition[a] (mean)
Esophagus (high-risk areas in China):	
Lin-xian	71
26 counties	48
69 counties	63
Stomach (high-risk areas) in:	
Japan	75
Poland	58
Costa Rica (children)	22
Nasopharynx (high-risk area in southern China)	55
Liver:	
Cirrhotic patients (Europe)	67
Fluke-infested (Thailand)	80
Aerodigestive tract (smokers)	62

[a] Data are calculated from urinary NPRO after dosing subjects po with proline with and without ascorbic acid. (From Ref. 13.)

hydroxylation, is a well-recognized metabolic activation process for many nitrosamines. This is illustrated for dialkylnitrosamines in Figure 2. The resulting product is an α-hydroxydialkylnitrosamine, which can be generated *in situ* by hydrolysis of the corresponding α-acetoxynitrosamine. α-Hydroxynitrosamines have a half-life of up to 10 sec under physiologic conditions (32). They spontaneously decompose to an aldehyde and a diazohydroxide. The latter dissociates to a diazonium hydroxide and ultimately to a carbocation, depending on the nature of the R group. The diazohydroxide and subsequent intermediates are highly electrophilic. Their major reaction is with H_2O, giving an alcohol, but they also react with DNA to produce a variety of alkylated DNA bases. Detoxification by denitrosation competes with this metabolic activation process (33). The denitrosation is also catalyzed by cytochromes P450 and results ultimately in the production of nitrite, an aldehyde, and a primary amine.

The reaction pathways illustrated in Figure 2 have been most extensively studied for NDMA (R = H). Cytochrome P450 2E1 is the major enzyme involved in the production of α-hydroxy NDMA, which fragments to yield formaldehyde

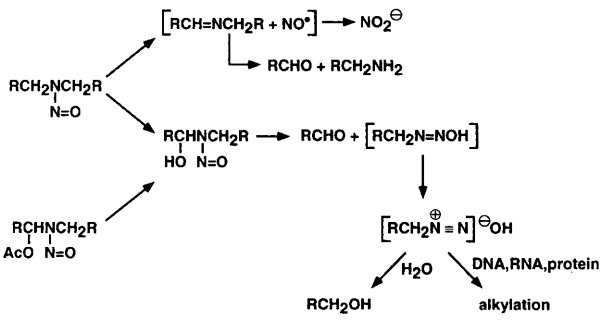

Figure 2. Metabolic α-hydroxylation and denitrosation of dialkylnitrosamines.

and the methanediazonium ion (34). The latter is typical of alkylating agents that react with DNA and RNA at a number of different sites, including N-7, N-3, N-1, N^2, and O^6 of guanine; N-3, O^2, and O^4 of thymidine or uridine; N-3, O^2, or N^4 of cytidine; N-1, N-7, N-3, and N^6 of adenosine, as well as the phosphate backbone (35, 36). Upon administration of NDMA to rats, 7-methylguanine is quantitatively the most significant adduct formed with DNA bases, but O^6-methylguanine, produced initially in one-tenth the amount as 7-methylguanine, is believed to be responsible for the carcinogenic effects of NDMA. This adduct is promutagenic, causing GC → AT transition mutations (37, 38). A DNA repair protein, O^6-alkylguanine-DNA-alkyltransferase, can remove the methyl group from O^6-methylguanine, reconverting it to guanine (39). When this repair protein is depleted, O^6-methylguanine can accumulate, resulting in critical mutations in oncogenes and tumor suppressor genes. Such gene changes lead to derangement of normal cellular growth control processes, and ultimately to cancer. Thus, formation of the methanediazonium ion *via* α-hydroxylation is the key step in NDMA metabolic activation; in the absence of this reaction, tumors will not be formed. α-Hydroxylation is also involved in NDMA hepatotoxicity (40). Denitrosation accounts for 15%–30% of NDMA metabolism and is thought to be a detoxification pathway (33, 40).

While cytochrome P450 2E1 is the major enzyme involved in NDMA metabolism, higher dialkynitrosamines are not efficiently oxidized by this enzyme. Instead, a variety of other cytochrome P450 enzymes are involved, with specificity depending on the nature of the alkyl chains (41, 42). Metabolic activation of NDEA (R = CH_3) proceeds by α-hydroxylation resulting in ethylation of DNA. O^2-Ethylthymine and O^4-ethylthymine are quantitatively minor adducts initially. However, they are repaired inefficiently, leading to their persistence and accumulation in hepatocyte DNA. They appear to be important in the induction of hepatocellular carcinoma by NDEA (43).

The metabolic activation of *N*-nitrosodipropylamine (R = C_2H_5) is more complex than that of NDMA and NDEA (31). This nitrosamine methylates DNA, in addition to the expected propylation reaction. The mechanism involves initial hydroxylation of the β-carbon, followed by oxidation to give *N*-nitoso-2-oxopropylpropylamine. α-Hydroxylation of the propyl group produces 2-oxopropyldiazotate, which rearranges to give the methylating agent diazomethane (31).

NNK is a tobacco-specific nitrosamine and a potent and selective lung carcinogen in the rat, independent of the route of administration. Thus, adenoma and adenocarcinoma of the lung are the major tumor types induced by NNK in the rat, whether it is administered by subcutaneous injection, in the drinking water, by oral swabbing, or by instillation in the bladder (8, 19). NNK is believed to play a significant role, along with polynuclear aromatic hydrocarbons, in the induction of lung cancer in smokers. The metabolism of NNK is summarized in Figure 3 (44). The presence of the

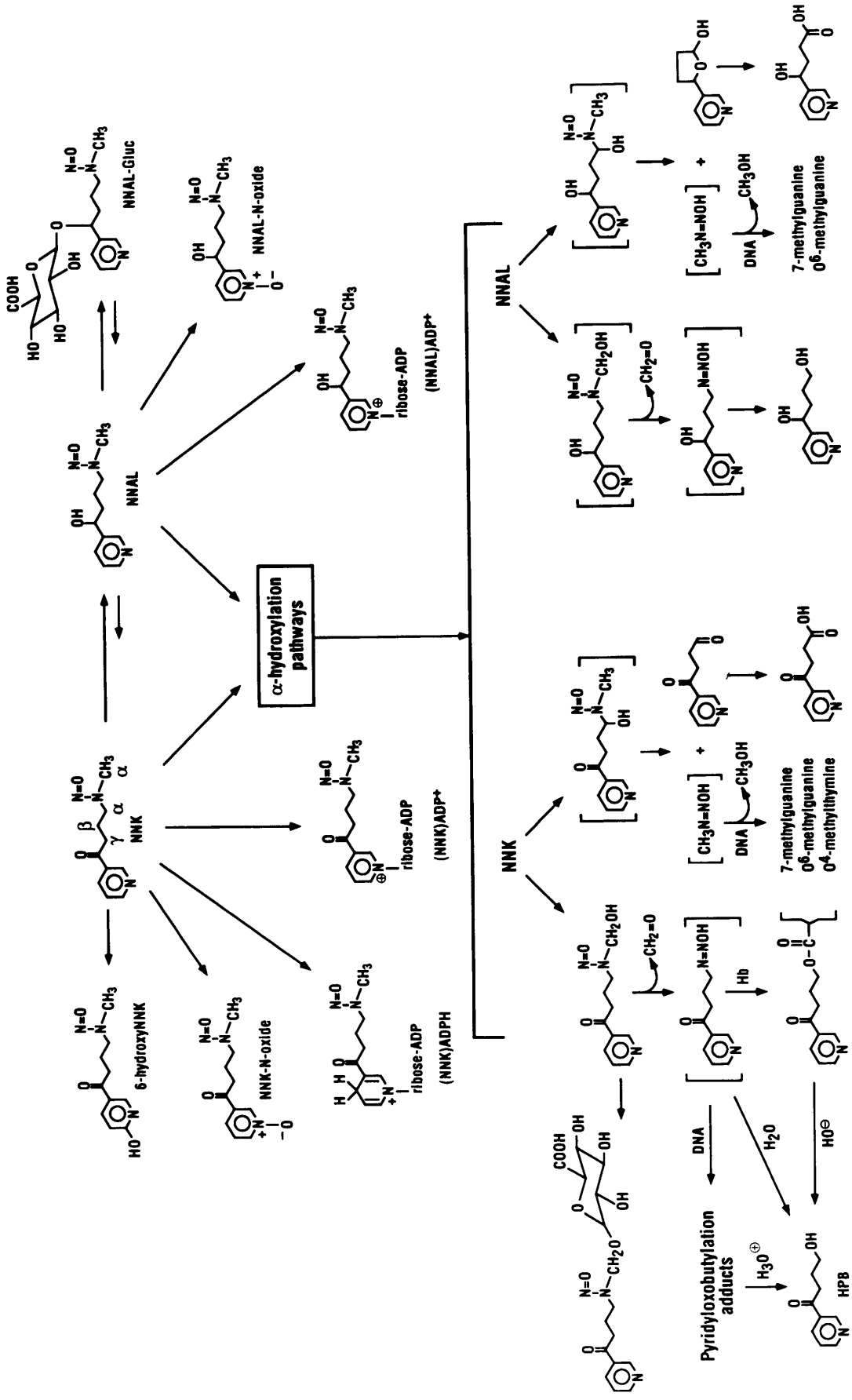

Figure 3. Metabolic pathways of NNK.

pyridine ring and carbonyl group lead to a somewhat more complex series of reactions than observed for simple dialkynitrosamines. The major pathway of NNK metabolism in laboratory animals and humans is reduction of the carbonyl group, catalyzed by carbonyl reductase enzymes such as 11-β hydroxysteroid dehydrogenase (45). The product, NNAL, is also a potent pulmonary carcinogen and appears to be the major transport form of NNK (46, 47). Glucuronidation of NNAL produces NNAL-Gluc, which is excreted in the urine of laboratory animals and humans, and is believed to be a detoxification product of NNK (48–50). Other detoxification products are NNK-N-oxide and NNAL-N-oxide, resulting from cytochrome P450 catalyzed oxidation of the pyridine ring. An interesting series of products is formed *in vitro,* by substitution of NNK or NNAL for nicotinamide in NADPH, catalyzed by NAD^+ glycohydrolase (51). The α-hydroxylation pathways of NNK, catalyzed by cytochromes P450, result in intermediates that methylate and pyridyloxobutylate DNA. The strong pulmonary tumorigenicity of NNK is associated with the formation and persistence of these adducts in the Clara and type II cells of the rat lung (52, 53).

Chemoprevention of Nitrosamine Carcinogenesis by Agents that Alter Their Metabolism

If nitrosamine exposure cannot be prevented, chemoprevention of nitrosamine carcinogenesis is a viable alternative for diminishing cancer risk. Most chemoprevention strategies against nitrosamine induced cancer have been based on modification of nitrosamine metabolism. Any compound that can block the metabolic activation steps, scavenge the reactive intermediates, or enhance detoxification would be potentially a chemopreventive agent. Studies carried out to date have identified a number of compounds that inhibit nitrosamine metabolic activation, and some of these are also inhibitors of nitrosamine carcinogenesis. In many cases examined to date, the inhibitory effect appears to be due mainly to inhibition of the cytochrome P450 enzyme(s) involved in the α-hydroxylation metabolic activation step, but some agents may have multiple effects on the carcinogenic process. Examples of chemopreventive agents that inhibit nitrosamine metabolism and/or tumorigenicity include arylalkyl isothiocyanates, diallyl sulfide and related compounds, disulfiram and dithiocarbamates, ellagic acid, green and black tea, and *d*-limonene (54–68). Inhibition of tumorigenicity has been observed in the rat esophagus (NMBA), hamster pancreas (BOP), rat bladder (BHBN and *N*-nitrosobutyl-3-carboxypropylamine), mouse lung and forestomach (NDEA), and other sites. The extent of inhibition depends strongly on the precise structure of the inhibitor, the structure of the nitrosamine, and the target site.

Chemoprevention of NNK carcinogenesis has received a great deal of attention because of its central role as a tobacco-smoke carcinogen, and its likely involvement in lung cancer induction in smokers. As discussed above, reduction of NNK levels in tobacco products has not occurred. Although the best way to avoid exposure to NNK is to avoid tobacco products, many smokers cannot stop because of the addictive properties of nicotine. Agents tested for chemoprevention of NNK-induced lung tumorigenesis are summarized in Table II. This table includes only defined compounds, but it should be noted that certain mixtures such as tea, snuff extract, orange oil, and NIH-07 diet also inhibit NNK-induced tumorigenesis (81, 87, 91–93). Based on presently available data, isothiocyanates appear to be the strongest inhibitors of pulmonary carcinogenesis by NNK. Among these, phenethyl isothiocyanate (PEITC) is the most extensively studied.

PEITC ($PhCH_2CH_2N=C=S$) is a naturally occurring compound found as its conjugate, gluconasturtiin, in several cruciferous vegetables including watercress (94). PEITC is released from the vegetable upon chewing by the action of myrosinase, a thioglucosidase present in the plant. In three separate studies, PEITC added to NIH-07 diet at a concentration of 498 ppm (3 μmol/g diet), before and during treatment of rats with NNK, caused significant reductions in lung tumorigenicity; complete abolition of tumorigenesis was observed in two of these studies (74–76). Similar strong inhibitory effects have been observed in mice (69, 71–73). Other studies have shown that isothiocyanates with higher lipophilicity and lower reactivity with glutathione than PEITC are even stronger inhibitors of NNK tumorigenesis in mice (78).

The mechanism of inhibition of NNK carcinogenesis by PEITC has been examined (53, 76, 95–98). The major effect of PEITC is inhibition of the cytochrome P450 enzymes in the rat lung, which catalyze the critical α-hydroxylation pathways illustrated in Figure 3. This inhibition occurs without apparent toxicity and is persistent in long-term studies carried out under the chronic dosing protocols that have been employed to demonstrate chemoprevention of lung tumors. Levels of pulmonary DNA adducts, particularly pyridyloxobutylation adducts in the target type II cells of the lung, decrease during PEITC administration. There is a concomitant decrease of hemoglobin adducts and an increase in urinary NNAL and NNAL-Gluc. Experiments *in vitro* have shown that PEITC is a competitive inhibitor of NNK metabolic activation in rat lung microsomes, with IC_{50} ranging from 150 to 210 n*M,* and in explants of rat lung.

There is some evidence that PEITC may have similar effects in smokers as it does in rats (99, 100). Smokers who consumed watercress, a rich source of PEITC, excreted more NNAL and NNAL-Gluc in their urine during the period of watercress consumption than during consumption of their usual diet. Levels of NNAL-N-oxide were not affected. The results suggest that PEITC inhibited the α-hydroxylation pathways of NNK, as observed in rats. This can be attributed to inhibition by PEITC of cytochrome P450 1A2, a human hepatic cytochrome P450 known to metabolize by α-hydroxylation (100). If similar effects are

Table II. Inhibition of NNK-Induced Lung Tumorigenesis[a]

Chemopreventive agent	Strain and species	Protocol type[b]	Result	Reference
Isothiocyanates (R–N=C=S)				
R=Phenyl	A/J mouse	Pre	No effect	69
Benzyl	A/J mouse	Pre/post	No effect	69, 70
Phenethyl	A/J mouse	Pre	Inhibition	69, 71–73
	A/J mouse	Post	No effect	70
	F344 rat	Pre-con	Inhibition	74–76
3-Phenylpropyl	A/J mouse	Pre	Inhibition	72, 73
4-Phenylbutyl	A/J mouse	Pre	Inhibition	72, 73
5-Phenylpentyl	A/J mouse	Pre	Inhibition	73
6-Phenylhexyl	A/J mouse	Pre	Inhibition	71, 73, 77
8-Phenyloctyl	A/J mouse	Pre	Inhibition	78
10-Phenyldecyl	A/J mouse	Pre	Inhibition	78
1,2-Diphenylethyl	A/J mouse	Pre	Inhibition	78
2,2-Diphenylethyl	A/J mouse	Pre	Inhibition	78
Allyl	A/J mouse	Pre	No effect	78
Hexyl	A/J mouse	Pre	Inhibition	78
2-Hexyl	A/J mouse	Pre	Inhibition	78
Dodecyl	A/J mouse	Pre	Inhibition	78
4-Oxo-4-(3-pyridyl)butyl	A/J mouse	Pre	No effect	72, 73
Sinigrin	F344 rat	Pre-con	No effect	79
Indole-3-carbinol	A/J mouse	Pre	Inhibition	80
D-Limonene	A/J mouse	Pre	Inhibition	81
Ellagic acid	A/J mouse	Pre-con	Inhibition	82, 83
Butylated hydroxyanisole	A/J mouse	Pre-con	Inhibition	82
Sulindac	A/J mouse	Pre-con	Inhibition	82, 84, 85
β-Carotene + retinol	A/J mouse	Pre-con	No effect	82
Sodium selenite	A/J mouse	Pre-con	No effect	82
Oltipraz	A/J mouse	Pre-con	No effect	84
Diallyl sulfide	A/J mouse	Pre	Inhibition	86
Epigallocatechin-3-gallate	A/J mouse	Pre-con	Inhibition	87
Caffeine	A/J mouse	Pre-con	Inhibition	87
Esculin	A/J mouse	Pre-con	No effect	83
Esculetin	A/J mouse	Pre-con	No effect	83
4-Hydroxy-1-phenyl-1-pentanone	A/J mouse	Pre	Inhibition	88
7-Hydroxy-1-phenyl-1-octanone	A/J mouse	Pre	Inhibition	88
4-Hydroxy-1-(2-thienyl)-1-pentanone	A/J mouse	Pre	No effect	88
4-Hydroxy-1-(3-pyridyl)-1-pentanone	A/J mouse	Pre	No effect	88
4-Ipomeanol	A/J mouse	Pre	No effect	88
Ibuprofen	A/J mouse	Pre-con	Inhibition	85
Piroxicam	A/J mouse	Pre-con	Inhibition	85
Naproxen	A/J mouse	Pre-con	No effect	85
1,4-Phenylenebis(methylene)selenocyanate	A/J mouse	Pre-con	Inhibition	89
4-Phenyl-1-butyne	A/J mouse	Pre	Inhibition	77
5-Phenyl-1-pentyne	A/J mouse	Pre	Inhibition	77
2-Ethynylnaphthalene	A/J mouse	Pre	Inhibition	77
myo-Inositol	A/J mouse	Pre-con	Inhibition	90
Dexamethasone	A/J mouse	Pre-con	Inhibition	90

[a] Only defined compounds are considered.
[b] Pre, compound given before NNK treatment; Pre-con, compound given before and during NNK treatment or before, during and after NNK treatment; post, compound given after NNK treatment.

occurring in human lung, PEITC may diminish DNA damage and cancer induction by NNK in smokers.

Summary

Nitrosamines are a large group of versatile carcinogens, inducing tumors at many important sites of human cancers. They are complete carcinogens, which readily cause tumors without need for promoting or cocarcinogenic agents. Nitrosamines are quite selective for tumor induction at specific sites, depending on their structures. They are metabolically activated to DNA-damaging species by a simple cytochrome P450-catalyzed hydroxylation step. There can be no question that humans exposed to sufficient amounts of nitrosamines would be susceptible to their carcinogenic effects. Human exposure does occur widely, although, with the exception of tobacco products, exposures have decreased due to preventive measures. Endogenous formation

of nitrosamines, however, can be extensive. Considerable evidence is available that nitrosamines are important causative agents for a number of different human cancer types including cancers of the oral cavity, lung, esophagus, pancreas, liver, nasopharynx, and bladder. There are three major cancer prevention strategies dictated by these facts. The first two, prevention of exposure to preformed nitrosamines and prevention of endogenous formation of nitrosamines, are the most desirable. Considerable progress has been made in reducing human exposure to preformed nitrosamines, except in tobacco products, where levels of exposure are still exceptionally high. The use of ascorbic acid and other inhibitors of nitrosation has shown great promise for reducing the endogenous formation of nitrosamines. The increased understanding of endogenous nitrosation mechanisms associated with the inducible nitric oxide synthase pathway has also provided new insights on preventive strategies. The third approach toward prevention of nitrosamine carcinogenesis is chemoprevention. Some very powerful chemopreventive agents have been discovered that are highly effective against a variety of nitrosamines. This approach has been extensively studied in connection with chemoprevention of lung cancer induced by the tobacco carcinogen NNK. In addicted smokers, who are exposed to substantial amounts of this carcinogen, chemoprevention may be a way to decrease the risk for lung cancer.

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