

富山医薬大医誌15巻1号 2004年

## REVIEW

# Carcinogenesis of the tobacco specific production, especially in NNK, 4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone

Hekmat Osman Abdel Aziz, Kazuhiro Nomoto, Yasuo Takano  
First Department of Pathology, Toyama Medical Pharmaceutical University

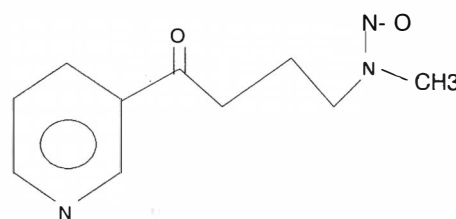
### Definition

NNK [4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone], a nicotine-derived nitrosaminoketone, is contained in tobacco smoke and is known to be a lung carcinogen. It is one of the most potent carcinogenic nitrosamines in laboratory animals and has, therefore, been implicated as a major cause of tobacco associated lung cancer<sup>(1)</sup>.

There are about 55 carcinogens 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<sup>(2)</sup>. NNK is a potent lung carcinogen in rats, mice, and hamsters<sup>(3)</sup>, being the only compound in cigarette smoke that has so far been found to induce 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 adenomas and adenocarcinomas, independent of the route of administration and in both susceptible and resistant strains of mice (Table 1)<sup>(3)</sup>. It has been proposed as partially responsible for the recent dramatic increase in adenocarcinoma of the lung<sup>(4)</sup>.

### Structure

Nicotine is a tertiary amine consisting of a pyridine and a pyrrolidine ring and NNN is formed by nitrosation of the latter. Production of NNK involves nitrosation under opening of the pyrrolidine ring (fig.2).



4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

Fig.2 structure of NNK

### Exposure

NNK 4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone has been found in a variety of tobacco products (chewing tobacco, snuff, cigarettes and cigars), in mainstream and side stream smoke from cigars and cigarettes, and in the saliva of chewers of betel quid with tobacco and oral snuff users. Daily exposure to tobacco specific nitrosamines is estimated as up to 20  $\mu$ g in smokers and 68  $\mu$ g in snuff dippers<sup>(5)</sup>.

### Experimental data

NNK was tested for carcinogenicity in several studies by subcutaneous injection in rats and hamsters and by intraperitoneal injection in mice. In hamsters, it induced benign and malignant tumors of the nasal cavity, trachea and lung, even after a single administration. In mice, NNK and its metabolites 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol, induced benign and malignant tumors of the lung. Both sensitive and resistant mouse strains develop lung tumors when treated with NNK, although the

incidence and multiplicity in resistant strains are lower and the tumors require a longer time to develop (Table. 1) <sup>(3)</sup>. The lung is the main target of NNK carcinogenicity although liver and forestomach tumors are seen occasionally. The A/J mouse, a sensitive strain, has been used extensively for studies of lung induction by NNK. The most commonly applied model features a single ip dose of 10  $\mu$  mol <sup>(6)</sup>. NNK and its metabolites can cross the placental barrier in mice and can be metabolically activated by mouse fetal tissues.

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

Species and strain	Route
Mouse	
A/J	i.p. gavage
Sencar	Skin
BALB/c	Oral
Swiss	Oral, i.p.
C3b6F1	i.p.
C3H/HeJ	i.p.
C57/BL/6	i.p.
(A/JxTSG-p53)F2	i.p.
F344 rat	s.c., p.o., oral swab, gavage, intravesicular
Syrian golden hamster	s.c., application to cheek pouch
Mink	s.c.

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

### Mechanisms of NNK induction of lung cancer

There are general principles for understanding the mechanism of tobacco carcinogenesis.

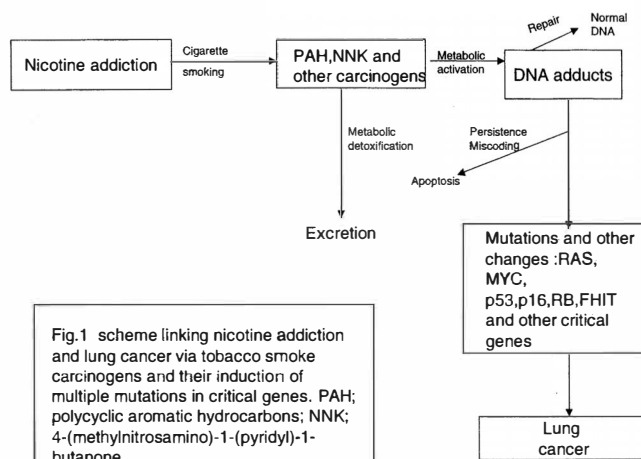
The following fig.(1) illustrates the overall framework for discussing these principles.

1. Carcinogens form the link between nicotine addiction and lung cancer.
2. Nicotine addiction is the reason that people continue to smoke <sup>(7)</sup>.
3. While nicotine itself is not considered to be carcinogenic, each cigarette contains a mixture of carcinogens, including small doses of polycyclic aromatic hydrocarbons (PAHs) and 4- (methylnitrosamino)-1-(3-

pyridyl)-1- butanone (NNK) among other lung carcinogens, tumor promoters and cocarcinogens <sup>(2)</sup>.

4. Carcinogens such as NNK& PAHs require metabolic activation to exert their effects; there are competing detoxification pathways, and the balance between metabolic activation and detoxification differs among individuals and will affect cancer risk.
5. The metabolic activation process leads to the formation of *DNA adducts, which are carcinogen metabolites bound covalently to DNA*, usually at adenine or guanine.
6. If DNA adducts escape cellular repair mechanisms and persist, they may lead to miscoding, resulting in a permanent mutation.
7. Cells with damaged DNA may be removed by apoptosis, or programmed cell death.
8. If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to a- activation of the oncogene or b- deactivation of the tumor suppressor gene.
9. Multiple events of this type lead to aberrant cells with loss of normal growth control and ultimately, to lung cancer.

There is now a large amount of data on mutations in 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 .



## NNK :uptake, metabolism, and adduct formation

Fewer than 20% of smokers will get cancer<sup>(8)</sup>. Susceptibility will depend in part on the balance between carcinogen metabolic activation and detoxification in smokers.

Carcinogens are enzymatically transformed to a series of metabolites as the exposed organism attempts to convert them to forms that are more readily excreted.

NNK metabolism pathways are illustrated in (fig. 3).

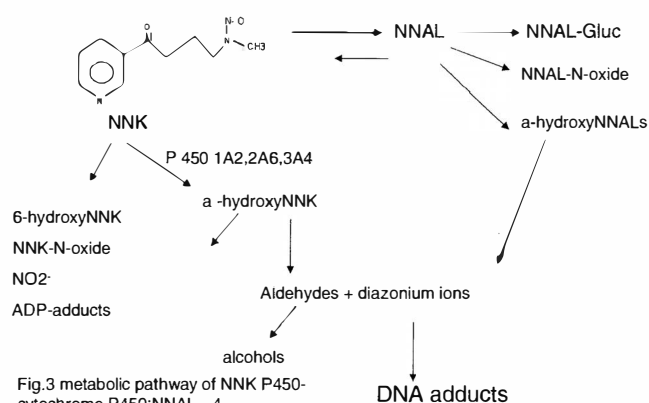


Fig.3 metabolic pathway of NNK P450-cytochrome P450; NNAL- 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

### 1. Metabolism of NNK and NNAL

Five types of transformations has been observed: Carboxyl reduction, Pyridine oxidation,  $\alpha$  - Methylene hydroxylation,  $\alpha$  - Methyl hydroxylation ADP adduct formation and Denitrosation.

- Carboxyl reduction;** reduction of the NNK carboxyl group produces NNAL<sup>(9)</sup>, the predominant NNK metabolite formed in vitro with many tissues. This is the case in rodent and human liver, as well as in human lung and in rat intestine. NNAL has carcinogenic activity similar to NNK and indeed can be oxidized to form NNK. NNAL can form NNAL-Gluc which is probably a detoxification product. P450 plays little role in NNAL formation.
- Pyridine oxidation;** pyridine oxidation results in the formation of NNAL N-oxide, the major metabolite of NNK in rat and mouse lung microsomes. Pulmonary P450s are the major catalysts of pyridine

n-oxidation as well as  $\alpha$  -hydroxylation of NNK and NNAL<sup>(10)</sup>.

- $\alpha$  - Methylene hydroxylation ;** hydroxylation of the methylene carbon adjacent to the N-nitroso group produces the unstable intermediate  $\alpha$  -methylenehydroxy-NNK, which spontaneously decomposes to methane diazohydroxide. This methylates DNA forming a variety of adducts.
- $\alpha$  - Methyl hydroxylation;** hydroxylation of the NNK methyl group yields  $\alpha$  -hydroxymethyl NNK which decomposes spontaneously to formaldehyde and diazohydroxide or diazonium ions. The latter pyriloxobutylate DNA and are important in NNK carcinogenesis.

### 2. Enzyme involvement in NNK metabolism

The initial steps are usually carried out by cytochrome P450 (P450s)

enzymes, encoded by the CYP family of genes, which oxygenate substrates<sup>(11)</sup>. Other enzymes such as lipoxygenases, cyclooxygenases, myeloperoxidase, and monoamine oxidases, may also be involved, but less frequently. P450s 1A2, 2A, 2B1, and 3A play roles in oxidative metabolism of NNK in rats and mice.

- Carboxyl reduction;** this reaction is mediated by carbonyl reductases. 11- $\beta$ -Hydroxysteroid dehydrogenase, a microsomal enzyme responsible for the interconversion of active 11-hydroxyglucocorticoids to inactive 11-oxo forms has been identified as one carboxyl reductase involved in the reduction of NNK to NNAL.
- Pyridine oxidation;** there is strong evidence that P450 2B1 is one of the major rat hepatic P450 forms responsible for conversion of NNK to NNK N-oxide.
- $\alpha$  - Methylene hydroxylation;** subtypes of P450s such as P450 1A1,2A1,2 B1,2F,2A4, 2A6 and 3A4, have been found to have a role in this reaction.
- $\alpha$  - Methyl hydroxylation;** this is probably a P450 mediated reaction.

### 3. DNA binding of NNK and NNAL

Some of the metabolites produced by the P450s react with DNA or other macromolecules to form covalent binding products known as adducts. Their generation is referred to as metabolic activation, other reactions being considered as detoxification pathways. (Fig 3).

1. The major metabolic activation pathway 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 ( $\alpha$ -hydroxylation), which leads to the formation of two types of adducts: methyl adducts, such as 7-methylguanine or O<sup>6</sup>-methylguanine, and pyridyloxobutyl adducts.

2. O<sup>6</sup>-methylguanine plays a critical role in mouse lung tumorigenesis by NNK<sup>(3)</sup>.

Thus there are two major types of NNK-DNA adducts: methyl adducts formed by  $\alpha$ -Methylene hydroxylation and pyridyloxobutyl adducts formed by  $\alpha$ -Methyl hydroxylation.

DNA methylation ( $\alpha$ -Methylene hydroxylation).  $\alpha$ -Methylene hydroxylation of NNK yields methane diazohydroxide and/or the methyldiazonium ion, which reacts with DNA producing 7-mG, O<sup>6</sup>-mG, and O<sup>4</sup>-mT. DNA methylation by NNK has been observed in a number of in vitro studies with different systems capable of metabolic activation, including rat lung cells and lung, liver, or nasal mucosal microsomes, rat oral tissue, and hamster lung.

DNA Pyridyloxobutylation ( $\alpha$ -Methyl hydroxylation). Pyridyloxobutyl adducts inhibit AGT, the enzyme responsible for repair of O<sup>6</sup>-mG.

#### DNA repair processes

DNA repair processes are important in determining whether DNA adducts persist. Because smoking is a chronic habit, we can expect a steady-state DNA adduct level to be achieved

by the opposing effects of damage and repair: direct repair, base excision repair and nucleotide excision repair. With respect to smoking and lung cancer, direct repair of O<sup>6</sup>-methyldeoxyguanosine by O<sup>6</sup>-methylguanine-DNA alkyltransferase (AGT) and nucleotide excision repair of PAH-DNA adducts would appear to be the most relevant processes. In smokers, the AGT that would repair O<sup>6</sup>-methylguanine formed from NNK, might be inhibited by pyridylobutylated DNA, as shown in mice<sup>(2)</sup>.

#### Effects of NNK on tumor suppressor genes and oncogenes.

As indicated in fig<sup>(1)</sup>, 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.

##### *The p53 gene*

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. Point mutations at G are common. In a sample of 550 p53 mutations in lung tumors, 33% were G→T transversions while 26% were G→A transitions. (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 non-transcribed DNA strand also has been noted. These observations are generally consistent with the fact that most activated carcinogens react predominantly at G and the repair of the resulting adducts would be slower on the nontranscribed strand, and thus support the hypothesis outlined in fig.1<sup>(3)</sup>.

Many factors influence the type of mutations

of p53. These include the type of DNA adduct formed, the extent to which it is repaired, its sequence context, and the DNA polymerases involved.

It has been demonstrated that cytosine methylation greatly enhances guanine alkylation at all CpG sites in the p53 gene by a variety of carcinogens. O<sup>6</sup>-Alkylguanine, formed from nitrosamines, is another likely cause of G→A transitions. NNK derived intermediates can cause G→T transversion.

#### Kras gene;

Mutations in codon 12 of *Kras* are found in 24%-50% of human primary adenocarcinomas but are rarely seen in other lung tumor types<sup>64</sup>. These mutations are more common in smokers and exsmokers than in non smokers, which suggests that they may be induced by direct reaction with the gene of an activated tobacco smoke carcinogen.

In the mouse, the O<sup>6</sup>-methylguanine pathway of NNK metabolic activation is dominant, resulting in a high percentage of GGT→GAT mutations in codon 12 of the *Kras* gene.

The p16<sup>ink4a</sup> 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.

In the rat, 94% of the adenocarcinomas induced by NNK have been found to be hypermethylated at the p16 gene promoter. This change is frequently detected in hyperplastic lesions and adenomas, which are precursors to the adenocarcinomas induced by NNK. Similar results were obtained for human squamous cell carcinomas of the lung. Methylation of p16 is 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 of lung cancer<sup>65</sup>.

#### Retinoblastoma (RB)

The expression of cell cycle proteins is related

to p16 and retinoblastoma (RB) genes; NNK-induced mouse lung tumors appear to resemble human non-small-cell lung cancers in cell cycle proteins<sup>66</sup>.

#### References

1. Hecht SS and Hofmann D, Tobacco-specific nitrosamines: an important group of carcinogens in tobacco smoke, *Carcinogenesis* 1988; 9: 1665-1668.
2. Hoffmann D and Hoffmann I, The changing cigarette, 1950-1995. *J Toxicol Environ Health* 1997;50:307-64.
3. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem Res Toxicol* 1998;11:559-603.
4. Westra WH, Slebos RJ, Offerhaus GJ, Goodman SN, Evers SG, Kensler TW, et al. K-ras oncogene activation in lung adenocarcinoma from former smokers. Evidence that K-ras mutations are an early and irreversible event in the development of adenocarcinoma of the lung. *Cancer* 1993; 72: 432-8.
5. Hoffmann D, Brunnemann K. D, Prokopczyk B, and Djordjevic, M V. tobacco specific n-nitrosamines and areca-derived n-nitrosamines: chemistry, biochemistry, carcinogenicity, and relevance to humans. *J. Toxicol. Environ. Health* 1994;41:1-52.
6. Hecht SS, Morse MA, Amin S, Stoner GD, Jordan KG, Choi CI, and Chung FL. Rapid single-dose model for lung tumor induction in A/J mice by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the effect of diet. *Carcinogenesis* 1989;10: 1901-1904.
7. Surgeon General. The health consequences of smoking: nicotine addiction. Washington (DC): U.S. Gov Print; 1988.
8. International Agency for Research on Cancer (IARC). Tobacco smoking. In: IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol 38. Lyon (France): IARC; 1986; 37-375.

9. Hecht, S, Young R, and Chen C B. Metabolism in the F344 rat of 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Cancer Res* 1980; 40: 4144-4150.
10. Ardies CM, Smith TJ, Kim S, and Yang CS. Induction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) activation in rat lung microsomes by chronic ethanol consumption and repeated running exercise. *Cancer Lett* 1996; 103:209-218.
11. Guengerich FP. Cytochrome P450 enzymes. In Guengerich FP, editor. *Comprehensive toxicology: biotransformation*. Oxford (U.K.): Elsevier Science; 1997; 3: 37-68.
12. Wang L, Spratt TE, Liu XK, Hecht SS, Pegg AE and Peterson LA. Pyridyloxobutyl adduct O6-(4-oxo-4-(3-pyridyl)butyl)guanine is present in 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone-treated DNA and is a substrate for O6-alkylguanine-DNA alkyltransferase. *Chem Res Toxicol* 1997; 10: 562-7.
13. Hussain SP and Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. *Cancer Res* 1998;85:4023-37.
14. Mills NE, Fishman CL, Rom WN, Wubin n and Jacobson DR. increased prevalence of K-ras oncogene mutations in lung adenocarcinoma. *Cancer Res* 1995; 55:1444-7.
15. United States Department of Health and Human Services. *The health consequences of involuntary smoking: a report of the Surgeon General*. Washington (DC): Department of Health and Human Services; 1986.
16. Issa JP, Baylin SB and Belinsky SA. Methylation of the estrogen receptor CpG island in lung tumors is related to the specific type of carcinogen exposure. *Cancer Res* 1996;56:3655-8.