Environmental Health Perspectives
Vol. 99, pp. 57–63, 1993

Tobacco-Specific Nitrosamine Adducts: Studies in Laboratory Animals and Humans

by Stephen S. Hecht,[1] Steven G. Carmella,[1] Peter G. Foiles,[1] Sharon E. Murphy,[1] and Lisa A. Peterson[1]

This paper describes quantitation of human hemoglobin and DNA adducts of the carcinogenic tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN). NNK and NNN are believed to be involved in cancers of the lung, esophagus, oral cavity, and pancreas in people who use tobacco products. The adduct dosimetry method employs GC–MS for quantitation of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) released by mild base hydrolysis of hemoglobin or acid hydrolysis of DNA as a biochemical marker of the pyridyloxobutylation metabolic activation pathway. Approximately 22% of smokers (n = 101) had elevated levels of HPB released from hemoglobin (range, 200–1600 fmole/g Hb). Adduct levels in snuff dippers ranged from 200–1800 fmole/g Hb. HPB levels in nonsmokers were generally below the detection limit. Acid hydrolysis of lung and tracheal DNA obtained at autopsy and analysis for released HPB revealed levels ranging up to 50 fmole/mg DNA in smokers; the adduct was not detected in nonsmokers. These findings are consistent with data generated in studies of adduct formation by NNK in rats. The biological significance of the HPB-releasing DNA pyridyloxobutylation pathway was compared to that of the DNA methylation pathway in the A/J mouse. These studies demonstrated that the persistence of O^6-methylguanine in lung DNA is critical for tumorigenesis by NNK and that pyridyloxobutylation enhances both persistence of O^6-methylguanine and tumorigenesis by acetoxymethylmethylnitrosamine. In the rat, the relative roles of methylation and pyridyloxobutylation in lung tumorigenesis by NNK are not as clearly defined. Although the biological significance of DNA methylation in NNK tumorigenesis is well characterized, dosimetry studies of tobacco-specific nitrosamines in humans should be carried out using biochemical markers of the pyridyloxobutylation pathway because of their specificity to tobacco products.

Introduction

Smoking is a well-established cause of cancer of the lung, larynx, oral cavity, esophagus, pancreas, and bladder (*1*). Oral use of smokeless tobacco causes oral cavity cancer (*2*). Tobacco users experience an intense and prolonged exposure to carcinogens and are therefore an appropriate group in which to assess carcinogen dosimetry. Among the various carcinogens present in tobacco smoke, three groups of compounds, polynuclear aromatic hydrocarbons, aromatic amines, and nitrosamines, appear to play major roles as causes of human cancers (*3*). Of the compounds present in unburned tobacco, nitrosamines are the most likely causes of cancer (*3,4*). Other papers in this issue describe dosimetry studies that have assessed polynuclear aromatic hydrocarbon and aromatic amine adduct levels in individuals exposed to tobacco smoke. In this paper, we focus on adducts of two tobacco-specific nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN). NNK and NNN have been implicated as causes of cancers of the lung, esophagus, oral cavity, and pancreas in tobacco users (*3,4*). This is based on their relatively high levels in tobacco products and on bioassays in laboratory animals that clearly demonstrate their tumorigenic activities in these tissues. We have developed methods to measure hemoglobin and DNA adducts of NNK and NNN in humans. We discuss these studies and describe parallel research in laboratory animals designed to increase our understanding of the measurements being made in humans.

[1]American Health Foundation, 1 Dana Road, Valhalla, NY 10595.
Address reprint requests to S. S. Hecht, American Health Foundation, 1 Dana Road, Valhalla, NY 10595.

Adduct Formation by NNK and NNN

Figure 1 shows the metabolic pathways that are known to form hemoglobin and DNA adducts of NNK and NNN. The metabolism of these compounds has been extensively studied in laboratory animals (*5–11*). Hydroxylation of the carbons adjacent to the nitroso nitrogen (α-hydroxylation) is the major pathway producing adducts. NNK is hydroxylated by cytochrome P-450 isozymes, and possibly by other mechanisms, to produce intermediates (1 and 2 in Fig. 1) (*11*). These intermediates are unstable and spontaneously decompose to either keto aldehyde (4 in Fig. 1) and methanediazohydroxide (5 in Fig. 1) or to formaldehyde (6 in Fig. 1) and 4-(3-pyridyl)-4-oxobutanediazohydroxide (7 in Fig. 1).

Methanediazohydroxide methylates globin to produce unknown adducts and methylates DNA, giving 7-methylguanine,

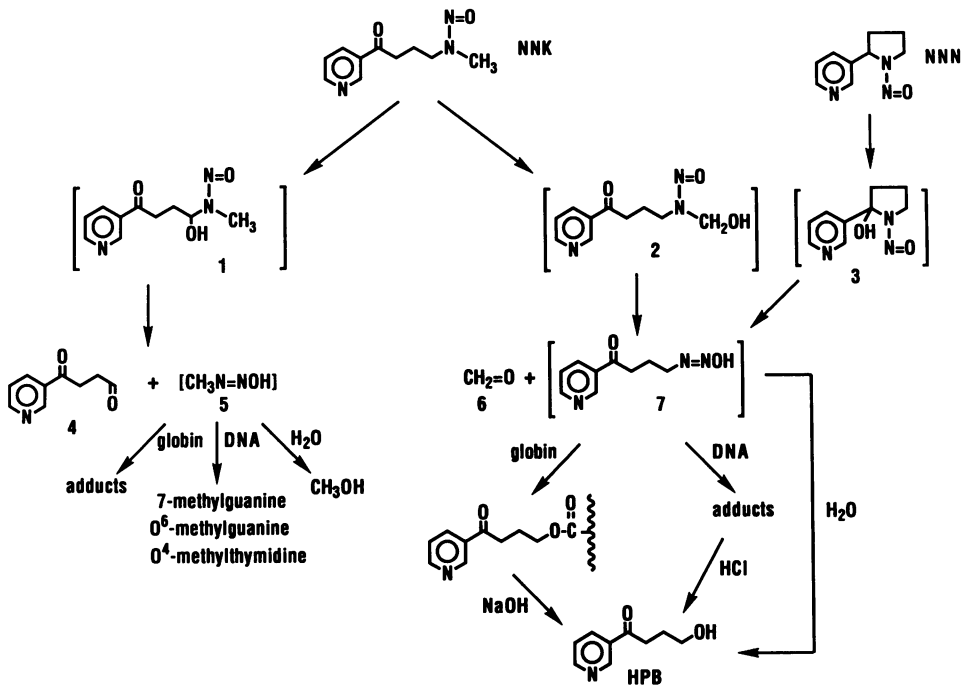

FIGURE 1. Metabolic activation of NNK and NNN to intermediates that bind to hemoglobin and DNA.

O^6-methylguanine, and O^4-methylthymidine. Other DNA adducts are probably formed, but they have not been identified in NNK-treated animals. Diazohydroxide (7 in Fig. 1) reacts with globin to produce a carboxylic ester adduct, which is readily hydrolyzed with base, yielding 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). Reaction of diazohydride with DNA, possibly through a cyclic oxonium intermediate (*12*), gives an adduct of unknown structure that is hydrolyzed to HPB with acid. Although other pathways of NNK metabolism such as pyridine-*N*-oxidation, pyridine ring oxidation, and carbonyl reduction have been well characterized, the pathways illustrated in Figure 1 appear to be the major reactions leading to adduct formation.

α-Hydroxylation of NNN at the carbon adjacent to the pyridine ring produces 2′-hydroxyNNN (3 in Fig. 1), which spontaneously decomposes to diazohydroxide (7 in Fig. 1), resulting in formation of the same globin and DNA adducts that are produced by methyl hydroxylation of NNK. As in the case of NNK, Figure 1 is an oversimplification. All carbons of the NNN pyrrolidine ring are hydroxylated, and the pyridine nitrogen is oxidized. None of these pathways have yet been associated with adduct formation.

These metabolism studies in laboratory animals established the basic patterns of NNK and NNN metabolism and adduct formation. The release of HPB from either globin or DNA by mild hydrolysis was especially attractive to us for use in dosimetry studies for several reasons. First, HPB could be readily separated from other constituents of globin or DNA by simple extraction. Second, HPB can easily be derivatized to improve its detectability. Third, HPB is structurally derived from NNK and NNN via diazohydroxide (7 in Fig. 1). Therefore, HPB should provide a specific marker for metabolic activation of NNK and NNN. This is not the case for DNA methylation by NNK since there are many other sources of human exposure to methylating agents.

Analysis of Hydrolysates of Human Hemoglobin or DNA for HPB

The analytical sensitivity required for the analysis of hydrolysates is in the parts per trillion range. This sensitivity is achieved by derivatization of HPB as its pentafluorobenzoate and quantitation by negative ion chemical ionization mass spectrometry. The detection limit for HPB-pentafluorobenzoate is approximately 100 amole. Specificity is achieved by the combination of capillary column gas chromatography and selected ion monitoring. Quantitation is accomplished using d_2-HPB as an internal standard, and HPB-tetrafluorobenzoate as an external standard. Methods for the analysis of HPB released from base hydrolysates of human hemoglobin or acid hydrolysates of human DNA have been published (*13,14*). Figure 2 summarizes the analytical scheme used for analysis of HPB released from hemoglobin; the method for DNA is similar except that the initial step is acid hydrolysis. Validation studies have shown that both methods are precise and reliable (*13,14*).

Figure 3 presents data obtained in ongoing analyses of HPB released from human hemoglobin. The samples were obtained from smokers, snuff dippers, and nonsmokers. Figure 3 illustrates several noteworthy points. First, HPB levels in smokers and snuff dippers are higher than in nonsmokers. This is consistent with the fact that NNK and NNN are derived from nicotine and are therefore associated only with nicotine-containing products such as tobacco and tobacco smoke. The mean level of HPB-releasing hemoglobin adducts in nonsmokers is lower than

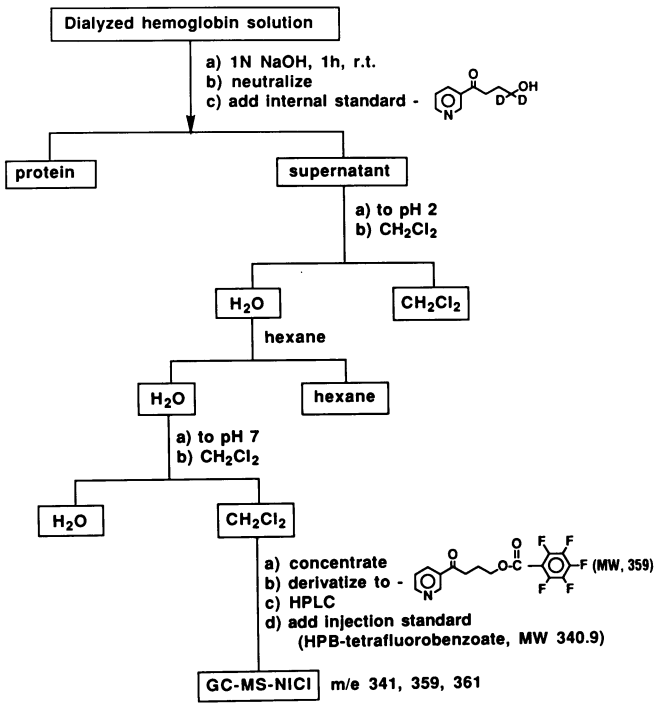

FIGURE 2. Scheme for the analysis of 4-hydroxy-1-(3-pyridyl)-1-butanone released from human hemoglobin.

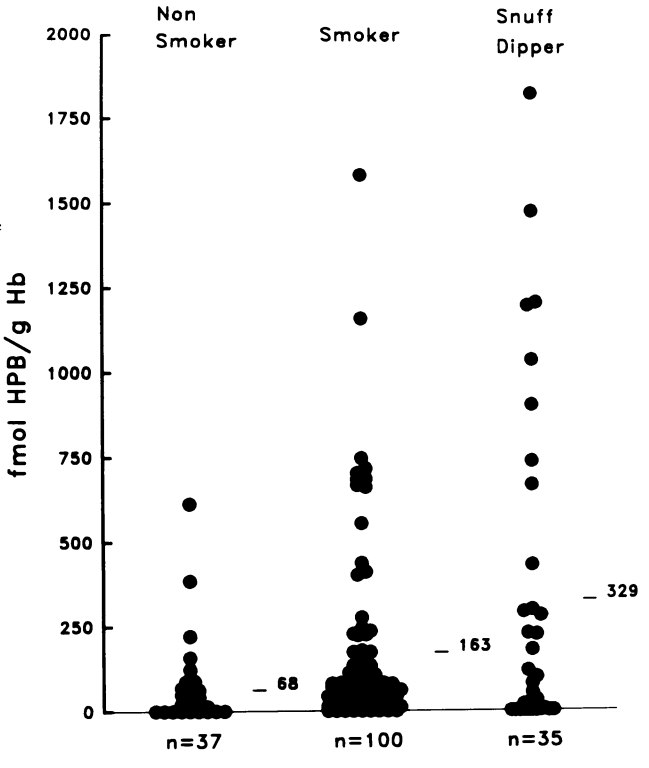

FIGURE 3. Levels of 4-hydroxy-1-(3-pyridyl)-1-butanone released from hemoglobin adducts of nonsmokers, smokers, and snuff dippers.

that of almost any other hemoglobin adduct that has been measured (*13*). Some of the lower nonsmoker values illustrated in Figure 1 may be due to methodological background. The comparisons between nonsmokers and tobacco users indicate that detection of HPB in hydrolysates of human hemoglobin should provide a reliable marker for chronic exposure to tobacco products. This may be important for studies of the relationship of environmental tobacco smoke to cancer.

Second, there is a wide spread in the HPB levels measured in both smokers and snuff dippers, ranging from not detected to approximately 1800 fmole/g Hb. This is consistent with *in vitro* studies in cultured human tissues, which have indicated that individuals differ in their capacity to metabolically activate NNK and NNN by the pathways illustrated in Figure 1 (*15*).

Several factors other than metabolic activation could contribute to high levels of the HPB-releasing adducts. The dose of NNK and NNN will be affected by an individual's smoking pattern or the extent of one's smokeless tobacco use. The nitrosamines may also be formed endogenously from nicotine or nornicotine. The persistence of the HPB-releasing hemoglobin adduct in humans is not well understood at present, although, based on studies in rats, the adduct appears to have a shorter lifetime than the erythrocyte. These factors require further study. Our hypothesis is that higher hemoglobin adduct levels will be associated with higher DNA adduct levels and consequently higher cancer risk. This is being tested in ongoing studies.

Third, only about 20% of smokers have HPB-releasing hemoglobin adduct levels that are higher than those in controls. This may relate to some of the factors discussed above. It will be important to determine what contributes to these high levels and their relationship to risk.

Fourth, snuff dippers have higher HPB-releasing hemoglobin adduct levels than smokers, at least among the individuals examined to date. The reasons for this are currently unknown but could relate to differences in enzyme induction patterns between snuff dippers and smokers. Perhaps constituents of tobacco smoke that are not present in snuff can induce the detoxification of NNK and NNN. Nevertheless, the results with snuff dippers support our hypothesis that NNK and NNN are involved in oral cancer induction because they show that snuff dippers can metabolically activate these carcinogens. NNK and NNN are by far the most abundant strong carcinogens in snuff, and they are the only carcinogens that have been shown to induce oral tumors in rats (*4,16*).

Further insight on the values in Figure 3 can be obtained by comparing the HPB values to those obtained upon chronic treatment of rats with NNK. Rats treated five times weekly for 5 weeks by IP injection of 0.5, 1, or 5 μg/kg NNK had 247 ± 5.9, 517 ± 3.2, or 1916 ± 153 fmole/g Hb of HPB-releasing adducts in their hemoglobin. These results suggest that the effective exposure of snuff dippers to diazohydroxide (7 in Fig. 1) was between 0.5 and 1 μg/kg/day. This is higher than the exposure calculated from levels of NNK and NNN in snuff, which would amount to approximately 0.1 μg/kg/day (*13*). Similarly, the results suggest a somewhat higher mean exposure of smokers to diazohydroxide (7 in Fig. 1) than would have been expected based only on calculations using levels of NNK and NNN in mainstream smoke (*13*). Overall, the levels of HPB-releasing adducts measured in humans were in the range expected based on the measurements made in rats treated with NNK. This supports the concept that the adducts being determined in humans do in fact result from exposure to NNK and NNN.

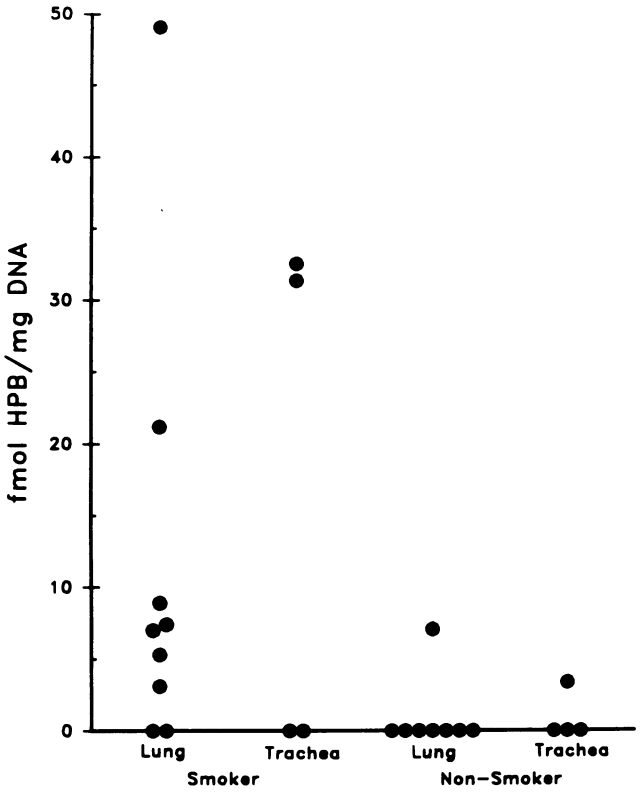

FIGURE 4. Levels of 4-hydroxy-1-(3-pyridyl)-1-butanone released from DNA adducts in peripheral lung and tracheobronchus of nonsmokers and smokers.

Figure 4 summarizes data obtained from analysis of human peripheral lung and tracheobronchus for levels of HPB released from DNA (*14*). The samples were obtained at immediate autopsy. The data show that HPB levels were higher in smokers than in nonsmokers, which is consistent with the hemoglobin analyses. The mean level of HPB released from peripheral lung DNA of smokers was 11,000 fmole/g DNA (0.016 μmole/μmole deoxyguanosine), about 70 times higher than the mean level released from globin of smokers (163 fmole/g Hb). In rats, at the lowest dose of NNK examined, HPB released from lung DNA was about 20 times greater than from hemoglobin when expressed per gram of macromolecule (*17*). These studies, taken together with those described above, show good agreement between the results in rats and humans.

When NNK was given in the drinking water to rats at a dose of 0.5 ppm for life, a significant incidence of lung and pancreatic tumors was induced when measured as part of a dose–response study (*18*). We estimate that the levels of HPB-releasing hemoglobin adducts in these rats would be about 8000 fmole/g Hb, or about 50 times higher than the mean level seen in smokers.

Relationship between Hemoglobin Adducts and DNA Adducts of NNK in Rats

With currently available methodology, it is more practical to quantify hemoglobin adducts than DNA adducts. In these studies, we wish to use HPB released from hemoglobin to estimate levels of HPB-releasing adducts in target tissue DNA and possibly to establish a relationship between levels of HPB-releasing adducts and levels of DNA methylation. These are complex issues which would best be addressed by quantifying all of these adduct levels in the same individuals. Approaches toward this goal are being developed. However, we have compared levels of pyridyloxobutylation and methylation in hemoglobin and DNA of rats treated with NNK.

Rats were treated with four IP doses of various amounts of NNK, labeled either in the methyl group or the pyridine ring, and sacrificed 4 hr after the last injection (*17*). Figure 5 illustrates the levels of total tritium bound to globin and of HPB released. There was a linear relationship between dose and tritium in globin over four orders of magnitude. The structures of the methyl adducts are not known, but they are not esters. Depending on dose, the adduct that releases HPB upon hydrolysis accounted for 17–40% of the bound tritium. This adduct has been identified as a carboxylic ester (*19*).

The relationships between DNA and globin pyridyloxobutylation and methylation are summarized in Figures 6 and 7. Whereas globin alkylation by both pathways increased linearly, levels of HPB released from lung or liver DNA and levels of 7-methylguanine in lung and liver DNA did not increase in a linear fashion. In lung, both pathways of DNA alkylation were more prevalent at lower doses than would have been expected by extrapolation from higher doses. This has also been observed for O^6-methylguanine in rat lung and Clara cell DNA and may relate to the presence of a high-affinity cytochrome P-450 form or to other factors (*20*). The ratio of 7-methylguanine to HPB released from lung DNA decreased with dose; it was 7.5 after four doses of 15 μg/kg/day of NNK. This suggests that these pathways may be quantitatively similar at the doses of NNK to which smokers are exposed.

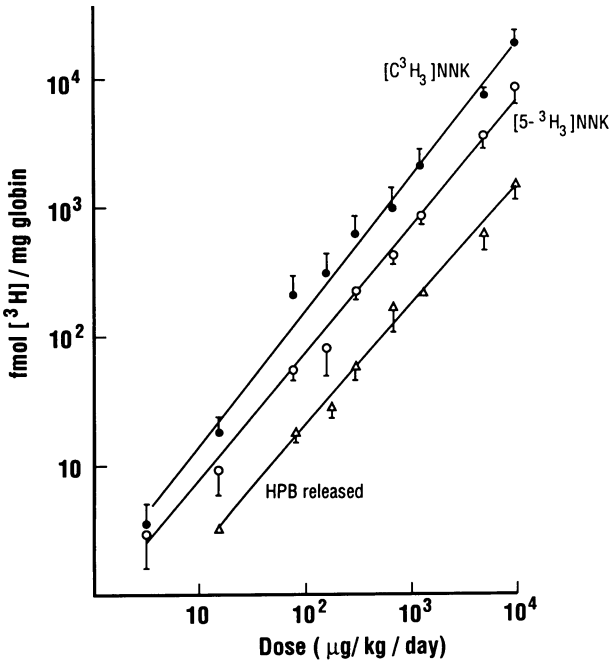

FIGURE 5. Log–log plot of the levels of tritium in rat globin after four daily IP injections (3–10,000 μg/kg) of either [C^3H_3]NNK or [5-^{3}H]NNK, and 4-hydroxy-1-(3-pyridyl)-1-butanone released from globin of the latter.

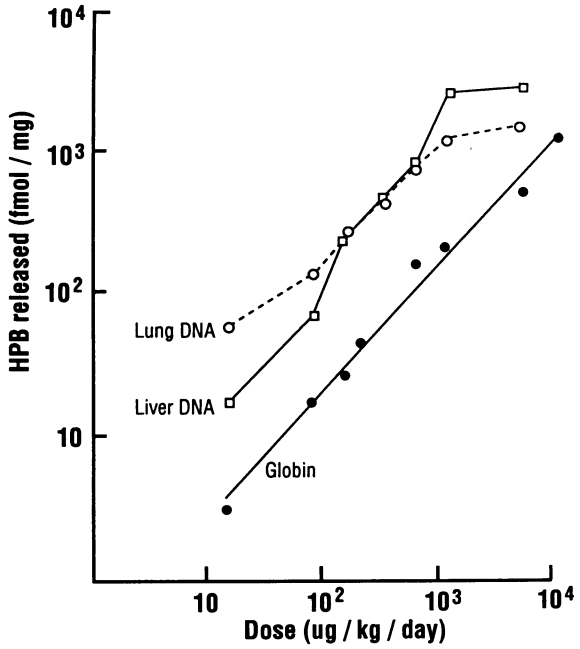

FIGURE 6. Log-log plot of 4-hydroxy-1-(3-pyridyl)-1-butanone released from globin after base hydrolysis and from lung and liver DNA after acid hydrolysis. Globin and DNA were obtained from rats treated with [5-^{3}H]NNK (3–10,000 μg/kg/day for 4 days).

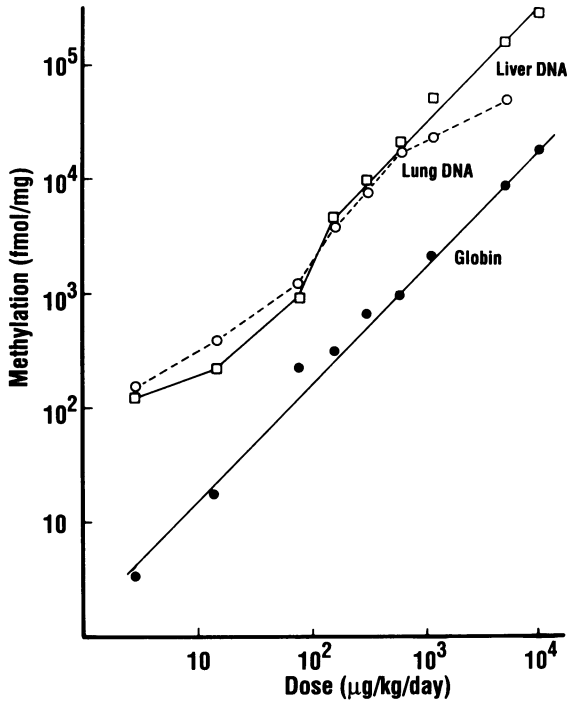

FIGURE 7. Log-log plot of levels of 7-methylguanine in lung and liver DNA and total tritium in globin after treatment of rats with [C^3H_3]NNK (3–10,000 μg/kg/day for 4 days).

Although levels of DNA adducts and hemoglobin adducts increase with dose, the nonlinear relationship between DNA alkylation and dose suggests that estimation of DNA adduct levels from hemoglobin adduct levels may be complex. There are several problems that need to be addressed before this can be confidently attempted. The mechanism of hemoglobin adduct formation is unclear. Presumably, the diazohydroxide is formed in metabolizing cells of the liver or perhaps lung and migrates into the erythrocyte where it reacts with globin. However, few data are available. If different cytochrome P-450 isozymes carry out methylene or methyl hydroxylation in different tissues, as seems likely, their contributions to globin adduct formation could vary widely. The contribution of DNA repair, which is known to occur for O^6-methylguanine, is another complication. Further studies assessing DNA and hemoglobin adduct levels in humans are required to resolve some of these issues.

Roles of DNA Methylation and Pyridyloxobutylation in Lung Tumor Induction by NNK

In A/J mice, NNK is an effective pulmonary carcinogen. We have developed a single-dose model for NNK tumorigenesis in the mouse lung, in which 10 μmole NNK given IP induces 8–10 lung tumors per mouse 16 weeks after injection (*21*). We have used this model to investigate the importance of DNA methylation and pyridyloxobutylation in mouse lung tumorigenesis. Substitution of deuteriums at the methylene carbon of NNK decreased levels of O^6-methylguanine in lung DNA and decreased tumorigenicity compared to NNK, whereas substitution of deuteriums at the methyl carbon had no major effect (*22*). These studies indicated that methylation was more important than pyridyloxobutylation in the induction of mouse lung tumors by NNK. This was consistent with the observation of Belinsky and co-workers (*23*) that a high percentage of mouse lung tumors induced by NNK had a G-C mutation in the second base of codon 12 of the K-*ras* gene, presumably as a result of the formation of O^6-methylguanine at this position.

To investigate the roles of methylation and pyridyloxobutylation more thoroughly, we have compared the DNA binding and tumorigenicity of acetoxymethylmethylnitrosamine (AMMN), a pure methylating agent, and 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNKOAc), a pure pyridyloxobutylating agent (*24*). DNA binding studies were carried out to determine doses of these compounds that would produce the same levels of methylated and pyridyloxobutylated DNA as did NNK. Then, the tumorigenic activities of these doses were determined.The results showed that AMMN was more tumorigenic than NNKOAc in mouse lung. Doses of AMMN that gave the same levels of persistent O^6-methylguanine in DNA as NNK had the same tumorigenicity as NNK. Interestingly, NNKOAc increased both the initial levels and persistence of O^6-methylguanine induced by AMMN, and combinations of a nontumorigenic dose of NNKOAc and AMMN were more tumorigenic than AMMN alone over a range of doses, as illustrated in Figure 8. Thus, pyridyloxobutylation appears to be a cocarcinogen for methylation, presumably by affecting the repair of O^6-methylguanine in mouse lung.

A striking correlation was observed between levels of O^6-methylguanine in mouse lung 96 hr after injection and the tumorigenic activities of AMMN, AMMN + NNKOAc, or NNK (Fig. 9). These results indicate that O^6-methylguanine formation is a critical determinant of mouse lung tumorigenesis induced by NNK.

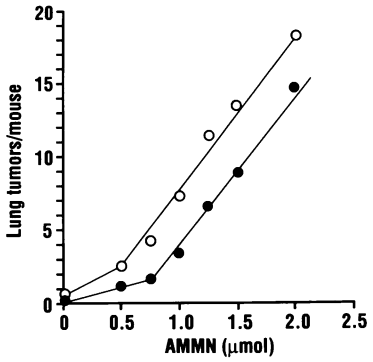

FIGURE 8. Dose response for lung tumor formation in A/J mice treated with acetoxymethylmethylnitrosamine (AMMN) ± 4.2 μmole 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNKOAc). The closed symbols are the mean values for groups receiving AMMN and the open symbols are those receiving AMMN + NNKOAc.

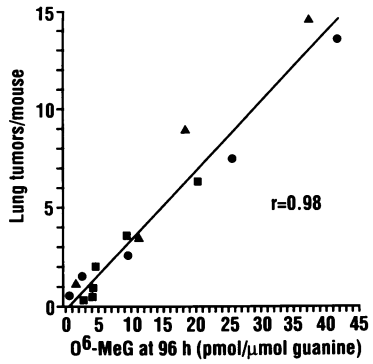

FIGURE 9. Relationship between tumor incidence and concentration of O^6-methylguanine (O^6-MeG) in lung DNA 96 hr after treatment with NNK (■), acetoxymethylmethylnitrosamine (▲), or acetoxymethylmethylnitrosamine + 4.2 μmole 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (●).

In the F344 rat, lung tumors are induced by chronic treatment with NNK, given either by SC injection or in drinking water (*6,18*). Deuterium substitution studies analogous to those described above were inconclusive (*25*). Neither substitution of deuterium at the methylene nor methyl carbon had any effect on tumorigenicity. Nevertheless, a dose–response study of O^6-methylguanine levels in Clara cell DNA and lung tumor incidence in rats treated with NNK showed a strong correlation between these two parameters, similar to that illustrated in Figure 9 (*20*). These results indicated an important role for O^6-methylguanine in lung tumor induction by NNK in the rat. If this were the case, perhaps the carcinogenicity of methylene deuterated-NNK should have been less than that of NNK; this was not observed. A further complication is that the tumors appear to arise from the alveolar type II cells of the lung in the rat, and no correlation between O^6-methylguanine and tumorigenicity was observed in these cells (*20*). In contrast to the A/J mouse, activated oncogenes have not been identified in the lungs of F344 rats treated with NNK (*26*). Thus, the relative roles of methylation and pyridyloxobutylation in the induction of tumors in the rat lung are not clear at present, although it appears that methylation is important in some way.

The uncertain role of pyridyloxobutylation in tumorigenesis is partly due to the present lack of structural data on the DNA adducts. Although these adducts are relatively stable in double-stranded DNA *in vitro* and *in vivo*, the major ones are apparently unstable at the nucleoside level, spontaneously producing HPB (*27*). This requires further investigation. Nevertheless, several lines of evidence support the concept that pyridyloxobutylation plays a role in tumorigenesis. First, NNN, which does not methylate DNA, is still an effective carcinogen, inducing tumors of the esophagus and nasal cavity in rats (*6*). Second, NNKOAc and a related model compound that generate diazohydroxide (7 in Fig. 1) upon hydrolysis are highly mutagenic in *S. typhimurium* strains, showing greater activity than AMMN. Consistent with this, methyl deuterated-NNK was not mutagenic, but both methylene deuterated-NNK as well as NNK alone were mutagenic toward *S. typhimurium* in the presence of an activating system (*28*). Third, studies of mutations induced by metabolically activated NNK in the *E. coli lac* I gene indicate the presence of a complex mutational spectrum consistent with both methylation and pyridyloxobutylation of DNA (*29*).

Although the role of DNA methylation in tumorigenesis by NNK is more clearly defined than that of pyridyloxobutylation, we believe that dosimetry studies of NNK should use biochemical markers that are specifically related to its structure, rather than those related to methylation. Sources of methylating agents such as dimethylnitrosamine are ubiquitous, and consequently methyl adducts are difficult to specifically trace to tobacco products.

Conclusions

We have developed methods to quantify HPB released upon mild acid or base hydrolysis of human DNA or hemoglobin. The HPB-releasing adducts are formed upon metabolic activation of the carcinogenic tobacco-specific nitrosamines NNK and NNN. Although the relationship of adduct levels to an individual's risk for developing tobacco-related cancers is not yet known, it is clear that certain individuals who use tobacco products have relatively high levels of these adducts compared to nonsmokers. The present methodology will contribute to our understanding of the metabolic activation of NNK and NNN in humans.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

This study was supported by grant nos. CA-29580 and CA-44377 from the National Cancer Institute.

REFERENCES

1. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 38. Tobacco Smoking. International Agency for Research on Cancer, Lyon, 1986, pp. 309–314.
2. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 37. Tobacco Habits Other Than Smoking: Betel-Quid and Areca Nut Chewing and Some Related Nitrosamines. International Agency for Research on Cancer, Lyon, France, 1985, pp. 113–116.
3. Hoffmann, D., and Hecht, S. S. Advances in tobacco carcinogenesis. In: Handbook of Experimental Pharmacology, Vol. 94-I (C. S. Cooper and P. L. Grover, Eds.), Springer-Verlag, Heidelberg, 1990, pp. 63–102.
4. Hecht, S. S., and Hoffmann, D. The relevance of tobacco-specific ni-

trosamines to human cancer. Cancer Surv. 8: 273–295 (1989).
5. Hecht, S. S., Castonguay, A., Rivenson, A., Mu, B., and Hoffmann, D. Tobacco specific nitrosamines: carcinogenicity, metabolism, and possible role in human cancer. Environ. Health Sci. 1(CI): 1–54 (1983).
6. Hoffmann, D., and Hecht, S. S. Nicotine-derived N-nitrosamines and tobacco related cancer: current status and future directions. Cancer Res. 45: 935–944 (1984).
7. Hecht, S. S., and Hoffmann, D. Tobacco-specific nitrosamines: an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis. 9: 875–884 (1988).
8. Belinsky, S. A., White, C. M., Trushin, N., and Hecht, S. S. Cell specificity for the pulmonary metabolism of tobacco-specific nitrosamines in the Fischer rat. Carcinogenesis 10: 2269–2274 (1989).
9. Peterson, L. A., Mathew, R., and Hecht, S. S. Quantitation of metabolic α-hydroxylation of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res. 51: 5495–5500 (1991).
10. Morse, M. A., Eklind, K. I., Toussaint, M., Amin, S. G., and Chung, F. L. Characterization of a glucuronide metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its dose-dependent excretion in the urine of mice and rats. Carcinogenesis 11: 1819–1823 (1990).
11. Smith, T. J., Guo, Z., Thomas, P.E., Chung, F. L., Morse, M. A., Eklind, K. I., and Yang, C. S. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung and its inhibition by isothiocyanates. Cancer Res. 50: 6817–6822 (1990).
12. Spratt, T. E., Peterson, L. A., Confer, W. L., and Hecht, S., S. Solvolysis of model compounds for α-hydroxylation of N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone: evidence for a cyclic oxonium ion intermediate in the alkylation of nucleophiles. Chem. Res. Toxicol. 3: 350–356 (1990).
13. Carmella, S. G., Kagan, S. S., Kagan, M., Foiles, P. G., Palladino, G., Quart, A. M., Quart, E., and Hecht, S. S. Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff-dippers, smokers, and nonsmokers. Cancer Res. 50: 5438–5445 (1990).
14. Foiles, P. G., Akerkar, S. A., Carmella, S. G., Kagan, M., Stoner, G. D., Resau, J. H., and Hecht, S. S. Mass spectrometric analysis of tobacco-specific nitrosamine DNA adducts in smokers and non-smokers. Chem. Res. Toxicol. 4: 364–368 (1991).
15. Castonguay, A., Stoner, G. D., Schut, H. A. J., and Hecht, S. S. Metabolism of tobacco-specific N-nitrosamines by cultured human tissues. Proc. Natl. Acad. Sci. U.S.A. 80: 6694–6697 (1983).
16. Hecht, S. S., Rivenson, A., Braley, J., DiBello, J., Adams, J. D., and Hoffmann, D. Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. Cancer Res. 46: 4162–4166 (1986).
17. Murphy, S. E., Palomino, A., Hecht, S. S., and Hoffmann, D. A dose response study of DNA adduct formation and hemoglobin adduct formation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats. Cancer Res. 50: 5446–5452 (1990).
18. Rivenson, A., Hoffmann, D., Prokopczyk, B., Amin, S., and Hecht, S. S. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and areca-derived N-nitrosamines. Cancer Res. 48: 6912–6917 (1988).
19. Carmella, S. G., Kagan, S. S., and Hecht, S. S. Evidence that a hemoglobin adduct used for dosimetry of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a carboxylic ester. Environ. Health Perspect. 99:203–205 (1993).
20. Belinsky, S. A., Foley, J. F., White, C. M., Anderson, M. W., Maronpot, R. R. Dose-response relationship between O^6-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res. 50: 3772–3780 (1990).
21. Hecht, S. S., Morse, M. A., Amin, S. G., Stoner, G. D., Jordan, K. G., Choi, C.-I., and Chung, F.-L. 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 10: 1901–1904 (1989).
22. Hecht, S. S., Jordan, K. G., Choi, C.-I., and Trushin, N. Effects of deuterium substitution on the tumorigenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in A/J mice. Carcinogenesis 11: 1017–1020 (1990).
23. Belinsky, S. A., Devereux, T. R., Maronpot, R. R., Stoner, G. D., and Anderson, M. W. Relationship between the formation of promutagenic adducts and the activation of the K-ras protooncogene in lung tumors from A/J mice treated with nitrosamines. Cancer Res. 49: 5305–5311 (1989).
24. Peterson, L. A., and Hecht, S. S. O^6-Methylguanine is a critical determinant of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone tumorigenesis in A/J mouse lung. Cancer Res. 51: 5557–5564 (1991).
25. Hecht, S. S., Lin, D., Castonguay, A., and Rivenson, A. Effects of α-deuterium substitution on the tumorigenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats. Carcinogenesis 8: 291–294 (1987).
26. Belinsky, S. A., Devereux, T. R., White, C. R., Foley, J. F., Maronpot, R. R., and Anderson, M. W. Role of Clara cells and type II cells in the development of pulmonary tumors in rats and mice following exposure to a tobacco-specific nitrosamine. Exp. Lung Res. 17: 263–278 (1991).
27. Spratt, T. E., Trushin, N., Lin, D., and Hecht, S. S. Analysis for N^2-(pyridyloxobutyl)deoxyguanosine adducts in DNA of tissues exposed to tritium labelled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N′-nitrosonornicotine. Chem. Res. Toxicol. 2: 169–173 (1989).
28. Hecht, S. S., Lin, D., and Castonguay, A. Effects of α-deuteriun substitution on the mutagenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Carcinogenesis 4: 305–310 (1983).
29. Jiao, J., Zielenska, M., Anderson, M. A., and Glickman, B. W. Mutational specificities of environmental carcinogens in the *lac* I gene of *Escherichia coli*: IV. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Carcinogenesis 12: 221–224 (1991).