Minor Tobacco Alkaloids as Biomarkers for Tobacco Use: Comparison of Users of Cigarettes, Smokeless Tobacco, Cigars, and Pipes

Peyton Jacob III, PhD, Lisa Yu, BS, Alexander T. Shulgin, PhD, and Neal L. Benowitz, MD

ABSTRACT

Objectives. This study (1) determined levels of various tobacco alkaloids in commercial tobacco products; (2) determined urinary concentrations, urinary excretion, and half-lives of the alkaloids in humans; and (3) examined the possibility that urine concentrations of nicotine-related alkaloids can be used as biomarkers of tobacco use.

Methods. Nicotine intake from various tobacco products was determined through pharmacokinetic techniques. Correlations of nicotine intake with urinary excretion and concentrations of anabasine, anatabine, nornicotine, nicotine, and cotinine were examined. By using urinary excretion data, elimination half-lives of the alkaloids were calculated.

Results. Alkaloid levels in commercial tobacco products, in milligrams per gram, were as follows: nicotine, 6.5 to 17.5; nornicotine, 0.14 to 0.66; anabasine, 0.008 to 0.030; and anatabine, 0.065 to 0.27. Measurable concentrations of all alkaloids were excreted in the urine of most subjects smoking cigarettes, cigars, and pipes and using smokeless tobacco. Correlations between nicotine intake and alkaloid concentrations were good to excellent.

Conclusions. Anabasine and anatabine, which are present in tobacco but not in nicotine medications, can be used to assess tobacco use in persons undergoing nicotine replacement therapy. (*Am J Public Health.* 1999;89: 731–736)

Tobacco and tobacco smoke contain numerous biologically active substances, including various alkaloids that are structurally related to nicotine.[1] Nicotine is the major pharmacologically active substance in tobacco. Most smokers are dependent on nicotine, and nicotine may play a role in the development of cardiovascular disease and reproductive disturbances.[2,3] Other tobacco alkaloids, when tested in animals, are pharmacologically active but less potent than nicotine—for example, nornicotine and anabasine.[4] Very little is known about the pharmacological effects of the minor tobacco alkaloids in humans. Anabasine administered orally or sublingually has been reported to aid smoking cessation and to have cardiovascular effects.[5] We are unaware of any other published accounts of studies of the human pharmacology of the minor tobacco alkaloids.

Exposure to nicotine-related alkaloids may also have toxicological significance. Some of the minor tobacco alkaloids are secondary amines and may be nitrosated during the storage of tobacco products or in vivo to give carcinogenic nitrosamines.[6] Nicotine and related alkaloids may be metabolized via chemically reactive intermediates, which may be capable of alkylating and altering the function of macromolecules.[7–9]

Concentrations of nicotine and its metabolites (primarily cotinine) in biological fluids are frequently used to ascertain whether or not a person is using tobacco and to estimate nicotine intake.[10–14] However, nicotine and its metabolites cannot be used to assess tobacco use in persons using nicotine gum, transdermal patches, nicotine inhalers, or other nicotine medications. Since minor alkaloids are present in tobacco but not in nicotine-containing medications, their measurement in biological fluids could be useful for detecting tobacco use in persons undergoing nicotine replacement therapy.

Nornicotine, anatabine, and anabasine are the most plentiful of the minor alkaloids in tobacco.[15] Nornicotine is also a minor metabolite of nicotine in humans.[16] This study reports on the levels of the alkaloids nornicotine, anabasine, and anatabine, as well as nicotine and cotinine, in the tobacco of various tobacco products and in the urine of persons using such products. Levels of these alkaloids were compared with nicotine intake, an index of tobacco consumption, to assess their usefulness as markers for tobacco use or exposure. Half-lives based on rates of urinary elimination of anabasine, anatabine, and nornicotine were also determined.

Methods

Subjects

Thirty-four healthy men, aged 20 to 62 years, who were habitual cigarette smokers, smokeless tobacco users, cigar smokers, or pipe smokers were recruited through newspaper advertisements. The 12 cigarette smokers (1 Black, 10 Whites, 1 Hispanic) smoked an average of 28 cigarettes per day (range = 15–40). Their screening plasma cotinine levels averaged 236 ng/mL (range = 72–497 ng/mL). The 9 smokeless tobacco users (3 Blacks, 6 Whites) used popular brands of oral snuff (Copenhagen or Skoal) or chewing tobacco

The authors are with the Division of Clinical Pharmacology, Department of Medicine, San Francisco General Hospital Medical Center, and the Department of Medicine and Drug Dependence Research Center, Langley Porter Psychiatric Institute, University of California, San Francisco.

Requests for reprints should be sent to Peyton Jacob III, PhD, University of California, San Francisco, San Francisco General Hospital, Bldg 100, Room 235, San Francisco, CA 94110 (email: peyton@itsa.ucsf.edu).

This paper was accepted November 23, 1998.

TABLE 1—Concentrations of Alkaloids in Commercial Tobacco Products[a]

Alkaloid	Type of Tobacco				
	Cigarette (13 Brands)	Oral Snuff (4 Brands)	Chewing (3 Brands)	Pipe (3 Brands)	Cigar (5 Brands)
Nicotine, mg/g (SD)[b]	17.5 (2.20)	13.5 (4.07)	6.49 (3.27)	14.4 (2.10)	9.13 (0.822)
Nicotine, % of total[c]	96.2	97.9	96.5	97.0	91.9
Nornicotine, mg/g (SD)[b]	0.382 (0.071)	0.173 (0.034)	0.140 (0.016)	0.199 (0.044)	0.658 (0.4)
Nornicotine, % of total[c]	2.11	1.32	2.35	1.37	6.54
Anabasine, mg/g (SD)[b]	0.030 (0.0039)	0.017 (0.0025)	0.0085 (0.0020)	0.029 (0.0081)	0.029 (0.0030)
Anabasine, % of total[c]	0.16	0.13	0.14	0.20	0.29
Anatabine, mg/g (SD)[b]	0.271 (0.034)	0.084 (0.024)	0.0650 (0.024)	0.214 (0.068)	0.127 (0.036)
Anatabine, % of total[c]	1.49	0.62	1.01	1.48	1.28

[a]Analyses were run in duplicate from domestic cigarettes, oral snuff, chewing tobacco, pipe tobacco, and cigars purchased in retail stores in the San Francisco area.
[b]Alkaloids were extracted from weighed portions of tobacco, and concentrations of alkaloids in the extracts were used to calculate concentrations in milligrams of alkaloid per gram of tobacco.
[c]Concentrations of the alkaloids were used to calculate levels of each as a percentage of the total alkaloids.

(Red Man). The screening cotinine levels in these subjects averaged 230 ng/mL (range = 10–436 ng/mL). The 5 pipe smokers (3 Blacks, 2 Whites) smoked 5 brands of tobacco (Captain Black, Borkum Riff, Mixture 79, Cherry Blend, or Drum) and had an average screening plasma cotinine level of 233 ng/mL (range = 17–442 ng/mL). The 8 cigar smokers (3 Blacks, 5 Whites) smoked an average of 5 (range = 2–10) small cigars daily (Garcia y Vega, Tijuana Smalls, Tiparillo, Swisher Sweets, or Wm. Penn, ranging from 90–125 mm in length, 10–12 mm in diameter, and 1.5–3.7 g in weight) and had an average screening plasma cotinine concentration of 397 ng/mL (range = 25–542 ng/mL). Written, informed consent was obtained after the procedures were fully explained. The study was approved by the Committee on Human Research at the University of California, San Francisco.

Experimental Protocol

Subjects were admitted to the General Clinical Research Center at San Francisco General Hospital for 5 days. During the first 3 days, the subjects were allowed to use their usual form of tobacco as desired. No tobacco use was permitted during the last 2 days. Urine was collected over 24 hours of each day for the first 3 days to measure tobacco alkaloid excretion. On the morning of the second day, subjects were given an intravenous infusion of deuterium-labeled nicotine (3',3'-dideuteronicotine, 2 μg/kg/min over 30 minutes). This infusion protocol produces peak blood levels of labeled nicotine comparable to those seen in subjects during cigarette smoking.[17] Blood samples were collected at 0, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240, 300, and 360 minutes to determine nicotine clearance (CL_{nic-d_2}). On the third day of each study block, while subjects continued ad libitum use of tobacco, blood samples were collected via an indwelling butterfly catheter every 2 hours from 8:00 AM until 12 midnight and at 4:00 AM and 8:00 AM the next morning. Plasma nicotine levels from samples collected during this period were used to determine nicotine intake from tobacco. On days 4 and 5, subjects were required to abstain from tobacco use. Urine was collected in 8-hour blocks to determine excretion rates and elimination half-lives of the various alkaloids.

Tobacco products in the categories of domestic cigarettes (13 brands), oral snuff (4 brands), chewing tobacco (3 brands), pipe tobacco (3 brands), and cigars (5 brands) were purchased from stores in the San Francisco Bay area and kept sealed in their original packages until the alkaloid content was analyzed.

Analytic Chemistry

Plasma and urine concentrations of nicotine, cotinine, and deuterium-labeled nicotine were determined by combined gas chromatography–mass spectrometry as described previously.[18] The cost of these assays was $50 per sample. Urine concentrations of anabasine, anatabine, and nornicotine were determined by a published gas chromatography–mass spectrometry method.[19] The cost of these assays was $80 per sample. Concentrations of alkaloids in tobacco products were determined by the above methods, after extraction from tobacco, by using a published procedure.[20] Samples were assayed in duplicate.

Data Analysis

Pharmacokinetic parameters for nicotine and cotinine were determined by model-independent methods.[13] The 24-hour dose of nicotine systemically absorbed from tobacco was determined from the area under the plasma concentration time curve for natural nicotine during ad libitum tobacco use on day 3 and from the clearance of labeled nicotine, as described previously.[17] Pearson correlation coefficients between intake of nicotine from tobacco with urine concentrations and 24-hour urinary excretion of alkaloids were computed by linear regression by using the software package Systat (version 8.0, SPSS Inc, Chicago, Ill). Elimination half-lives were determined by linear least squares regression of the log urinary excretion rate vs time curves. Comparisons of various parameters across different types of tobacco were made by analysis of variance.

Results

Levels of nicotine, nornicotine, anabasine, and anatabine in various commercial tobacco products are presented in Table 1. These products were not necessarily the same as those used by our subjects, but they are representative of the most popular American brands. Nicotine concentrations ranged from 6.5 to 17.5 mg/g and accounted for more than 90% of the total alkaloids in all forms of tobacco. The concentration of nicotine was lowest in chewing tobacco and highest in cigarette tobacco. As a percentage of the total alkaloids, nicotine in cigar tobacco was significantly less than in other forms of tobacco ($P < .005$). The concentrations of nornicotine were highest in cigar tobacco and lowest in chewing tobacco; the range was 0.17–0.66 mg/g. As a percentage of total alkaloids, nornicotine was also highest in cigar tobacco, with differences from other tobacco products significant ($P < .005$).

TABLE 2—Eight-Hour Urine Concentrations[a] and 24-Hour Excretion[b] of Tobacco Alkaloids During Ad Libitum Use of Various Tobacco Products by Human Subjects in a Research Ward

Alkaloid	Cigarette Smoking (n = 12)	Smokeless Tobacco (n = 9)	Cigar Smoking (n = 8)	Pipe Smoking (n = 5)
Anabasine, ng/mL (range)	6.20 (0.5–13.6)	6.33 (0.5–15)	2.84 (0.5–6.96)	5.87 (0.5–17.4)
Anabasine, μg/24 h (SD)	15.5 (11.5)	19.7 (18.2)	8.22 (6.67)	8.74 (8.68)
Anatabine, ng/mL (range)	11.5 (0.5–54.4)	14.9 (0.5–49.9)	2.84 (0.5–8.19)	4.60 (0.5–14.0)
Anatabine, μg/24 h (SD)	24.1 (25.3)	39.3 (40.2)	8.64 (8.03)	6.58 (6.25)
Nornicotine, ng/mL (range)	50.0 (3.39–121)	30.7 (1.74–98.2)	35.9 (3.4–88.4)	21.1 (0.5–46.3)
Nornicotine, μg/24 h (SD)	142 (108)	114 (165)	110 (95.7)	58.8 (67.8)
Nicotine, ng/mL (range)	1160 (100–3540)	732 (1–2530)	289 (20.0–820)	1107 (62.8–2530)
Nicotine, μg/24 h (SD)	2720 (2370)	1510 (1850)	896 (901)	1980 (2320)
Cotinine, ng/mL (range)	1280 (167–2380)	919 (122–2210)	670 (90.3–1210)	1358 (134–3610)
Cotinine, μg/24 r (SD)	3360 (2070)	2050 (2130)	1740 (1410)	3759 (5400)

[a]Urine was collected from 8 AM to 4 PM during ad libitum tobacco use. Concentrations were determined by gas chromatography–mass spectometry analysis of pooled urine collected during this period.
[b]Urine was collected during a 24-hour period of ad libitum tobacco use. Urinary excretion was determined from alkaloid concentrations and the volume of urine excreted.

Concentrations of anabasine ranged from 0.0085 to 0.029 mg/g and were lowest in the smokeless tobacco products. As a percentage of total alkaloids, anabasine was significantly higher in cigar tobacco than in the other tobacco products ($P < .001$). Anabasine as a percentage of the total was also significantly lower in oral snuff than in cigarette and pipe tobacco ($P < .05$). Anatabine concentrations ranged from 0.065 to 0.271 mg/g. Concentrations of anatabine were highest in cigarette tobacco and lowest in smokeless products. Anatabine as a percentage of the total alkaloids was significantly higher in cigarette tobacco than in smokeless tobacco products ($P < .005$). The percentage of anatabine in oral snuff was significantly lower than in other tobacco products (vs chewing tobacco, $P < .05$; vs all others, $P < .001$).

The mean concentrations and 24-hour urinary excretion of anabasine, anatabine, and nornicotine, as well as nicotine and its metabolite cotinine, in persons using various tobacco products ad libitum are presented in Table 2. The excretion of anabasine and anatabine was highest in smokeless tobacco users. The excretion of nicotine was highest in cigarette smokers, and the excretion of cotinine was highest in pipe smokers. Cigar smokers excreted the lowest levels of all alkaloids except nornicotine.

The intake of nicotine from persons using different tobacco products is given in Table 3. The daily intake of nicotine was highest in cigarette smokers, averaging 32.5 mg/day and with a range of 5.8–53.8 mg/day. The intake of nicotine was lowest in cigar smokers, averaging 14.6 mg/day.

The correlations between concentrations (8-hour collection) and urinary excretion (24-hour collection) of various alkaloids measured during ad libitum tobacco use and nicotine intake from tobacco were examined (Table 3). Generally, correlations of alkaloid levels with nicotine intake were good. For cigarette smokers, correlations of nicotine intake from tobacco with concentrations and with 24-hour urinary excretion of all 3 minor alkaloids, nicotine, and cotinine were statistically significant. The best correlations were for excretion of anabasine ($r = 0.79$, $P < .01$), nornicotine ($r = 0.80$, $P < .01$), and cotinine ($r = 0.97$, $P < .001$) (Table 3, Figure 1). Similar results were obtained for cigar smokers, smokeless tobacco users, and pipe smokers, although the correlations did not in all cases reach statistical significance. Correlations of nicotine intake with alkaloid levels determined for all subjects as a group, and for subjects using smoked tobacco grouped together, were highly significant.

The elimination half-lives of the alkaloids on the basis of urine excretion rates over 48 hours following cessation of tobacco use were as follows: anabasine, 15.9 hours (SD = 5.3, range = 10.1–26.8); anatabine, 9.6 hours (SD = 3.7, range = 5.8–15.4); nornicotine, 11.6 hours (SD = 4.2, range = 6.4–26.6); nicotine, 11.2 hours (SD = 6.3, range = 3.7–30.7); cotinine, 19.5 hours (SD = 7.0, range = 10.2–37.5).

Discussion

Our study presents several novel findings. First, we report levels of various tobacco alkaloids, including the minor alkaloids anabasine, anatabine, and nornicotine, in different types of commercial tobacco products. Second, urine concentrations and excretion data for these alkaloids in users of different tobacco products are presented, including new information on elimination half-lives. Third, systemic nicotine intake with regular use of cigars and pipe tobacco is reported for the first time. And fourth, urine levels of the minor alkaloids are shown to correlate well with systemic nicotine intake from various tobacco products, indicating the potential for use as biomarkers of tobacco intake in individuals using nicotine replacement medications.

As expected, nicotine was the major alkaloid in all tobacco products, but the concentration of nicotine varied considerably depending on the type. Concentrations were similar in cigarette, pipe, and oral snuff tobacco—approximately 1.5% by weight—but were only about half of this in cigar and chewing tobacco (Table 1). As a percentage of the total alkaloids, levels of anabasine were significantly lower in oral snuff than in the other tobacco products. Anabasine is a precursor for a carcinogenic nitrosamine, *N*-nitrosoanabasine. It has been reported that levels of tobacco-specific nitrosamines, including *N*-nitrosoanabasine, increase during the storage of smokeless tobacco products.[21] Conceivably, the lower levels of anabasine in oral snuff are due to its conversion to *N*-nitrosoanabasine and/or other degradation products during the curing of the tobacco.

Nicotine absorption, and the absorption of other substances from tobacco, depends not only on the amounts present in the tobacco consumed but also on how the tobacco is used. For smoked tobacco products, much of the nicotine and other substances are pyrolyzed or lost in sidestream smoke. There is considerable variability in smoking behavior, and smokers are able to control to a remarkable degree their nicotine absorption to achieve desired levels in the body.[20,22,23] With smokeless products, systemic absorption depends on how much is absorbed through buccal membranes and how

TABLE 3—Nicotine Intake[a] From Tobacco and Correlation of Nicotine Intake With 8-Hour Urine Concentrations[b] and 24-Hour Excretion[c] of Alkaloids by Human Subjects During Ad Libitum Tobacco Use in a Research Ward

Measure	Cigarette Smoking (n = 12)	Smokeless Tobacco (n = 9)	Cigar Smoking (n = 8)	Pipe Smoking (n = 5)	All Types Smoked Tobacco (n = 24)	All Subjects (n = 34)
Nicotine intake, mg/24 h (SD)	32.5 (16.3)	20.3 (14.4)	14.6 (12.3)	19.5 (18.0)	24.3 (16.9)	25.6 (20.7)
Nicotine intake, mg/24 h (range)	5.8–53.8	0.55–43.0	3.1–33.1	4.82–37.7	3.09–53.8	0.55–53.8
Anabasine concentration, *r*	0.70*	0.52*	0.70	0.69	0.69****	0.61****
Anabasine excretion, *r*	0.79**	0.47	0.94**	0.79	0.86****	0.61****
Anatabine concentration, *r*	0.62*	0.59*	0.69	0.65	0.64****	0.55****
Anatabine excretion, *r*	0.70*	0.62	0.86**	0.70	0.72****	0.52**
Nornicotine concentration, *r*	0.74**	0.36	0.81*	1.00****	0.77****	0.68****
Nornicotine excretion, *r*	0.80**	0.16	0.97****	0.80	0.79****	0.56***
Nicotine concentration, *r*	0.69*	0.73*	0.72*	0.89	0.75****	0.75****
Nicotine excretion, *r*	0.72**	0.76*	0.70	0.72	0.77****	0.77****
Cotinine concentration, *r*	0.80**	0.80**	0.80*	0.93	0.76****	0.77****
Cotinine excretion, *r*	0.97****	0.32	0.90**	0.97*	0.77****	0.70****

[a]Systemic nicotine intake from tobacco was determined through pharmacokinetic techniques during a 24-hour period of ad libitum tobacco use.
[b]See footnote a, Table 2.
[c]See footnote b, Table 2.
*$P < .05$; **$P = .01$; ***$P = .005$; ****$P < .001$.

much of what is swallowed escapes first-pass metabolism by the liver. For these reasons, a good estimate of absorption of nicotine or other toxic substances cannot be obtained by simply determining the amount of tobacco consumed.

We have previously described a methodology for determining nicotine intake from tobacco.[17,22] This methodology is analogous to the pharmacokinetic techniques used in drug bioavailablity studies. In the present study, the intake of nicotine was determined in human subjects by using various commercial tobacco products (Table 3). The systemic dose of nicotine (nicotine intake) can be used as an index of an individual's exposure to tobacco-derived toxins that are absorbed into systemic circulation.

We have previously reported the daily intake of nicotine from cigarette smoking and the use of smokeless tobacco.[22,24] We report here for the first time nicotine intake from cigar smoking and pipe smoking. The daily intake of nicotine, averaging 15 mg and 20 mg per day for the 2 groups, respectively, is similar to that taken in by typical cigarette smokers and smokeless tobacco users. Our findings are consistent with observations in a small study of plasma nicotine levels in smokers of small or large cigars[25,26] and plasma cotinine levels in regular pipe smokers.[27]

Estimating systemic exposure to nicotine and other toxins from tobacco is of interest to epidemiologists studying the risks of using various tobacco products and the hazards of exposure to environmental tobacco smoke. Such determinations are also of interest in the development of tobacco cessation treatments, since an objective measure of treatment outcome is needed. Measuring the levels of nicotine and its metabolite cotinine in biologic fluids is commonly used to quantitate tobacco use,[10–12] and levels of these substances have been correlated with nicotine intake.[14] However, nicotine and cotinine cannot be used to assess tobacco use in persons using nicotine-containing medications. Since the alkaloids anabasine and anatabine are present in tobacco but are not present in nicotine-containing medications and are not metabolically derived from nicotine, these alkaloids should be useful for estimating tobacco consumption during nicotine replacement therapy. To assess the utility of minor alkaloid measurement as a quantitative measure of tobacco use, we examined the correlation between concentrations and 24-hour urinary excretion of anabasine and anatabine—as well as of nornicotine, nicotine, and cotinine—during ad libitum tobacco use and nicotine intake (Table 3).

Most correlations between nicotine intake and levels of anabasine and anatabine in the urine of subjects using various tobacco products were high, with correlation coefficients (*r*) ranging from 0.47 to 0.94 (Table 3), although the small sample size for users of some of the tobacco products may limit the generalizability of these results. The concentrations and excretion rates of nicotine and cotinine correlated well with nicotine intake from cigarettes, as we have reported previously,[14] as well as with nicotine intake from smokeless tobacco use and cigar and pipe smoking, which has not been previously reported. Our data provide a basis for the use of measurements of concentrations of anabasine and anatabine in urine for estimating the extent of tobacco use during treatment with nicotine-containing medications. Of note, we have found that persons using nicotine gum but abstaining from tobacco do not excrete measurable amounts of anabasine or anatabine, confirming that nicotine gum does not contain significant levels of these alkaloids (unpublished results).

The sensitivity for detecting occasional tobacco use will depend not only on the analytic sensitivity for measuring anabasine and anatabine levels but also on the rate of elimination of these substances from the body. Consequently, we determined the half-lives of anabasine and anatabine from urinary excretion data obtained during tobacco abstinence. Both substances have relatively long half-lives, about 16 hours for anabasine and about 10 hours for anatabine. We are aware of only 1 other study of the elimination rate of minor alkaloids in humans. Beckett et al. reported data in 2 individuals given oral anabasine and nornicotine.[28] Half-lives of 4 hours were reported for each alkaloid. Our data, in contrast, are derived from a much larger number of subjects, a much longer urine collection period, and the use of a more sensitive assay methodology, which would explain the longer half-lives found in our study. Therefore, with a limit of quantitation of 1 ng/mL, levels should be detectable for 1 to 2 days following smoking cessation in a typical cigarette smoker, and somewhat longer in persons using smokeless tobacco.

Of interest is the finding that the elimination half-life of nicotine as based on urine excretion data averaged 11 hours. This is much longer than the half-life of 2 to 3 hours based on plasma concentrations.[14,17] The likely explanation is the slow release of nicotine from high-affinity tissue-binding sites.

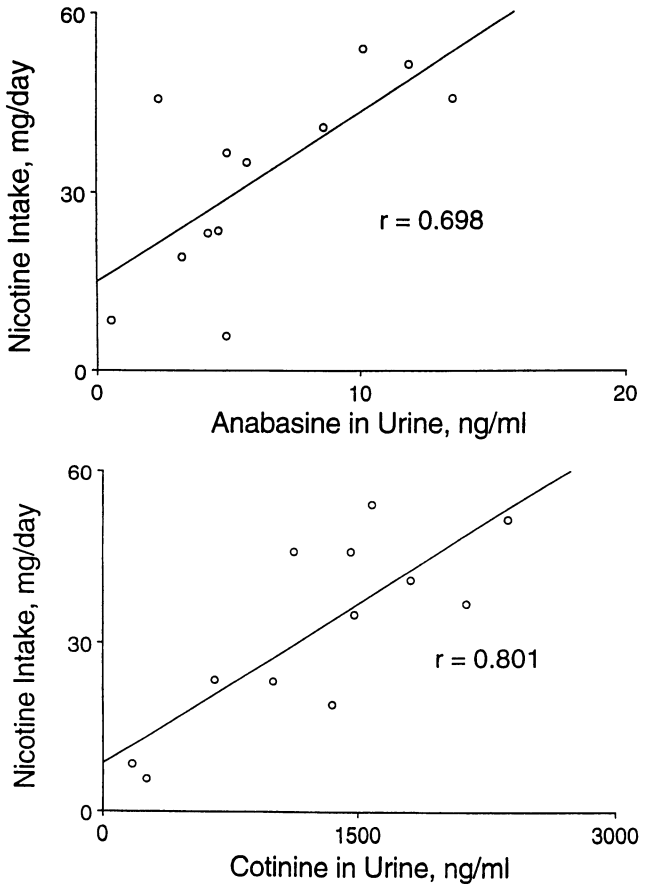

FIGURE 1—Correlations of nicotine intake with anabasine (upper panel) and cotinine (lower panel) concentrations in urine of 12 human subjects smoking cigarettes ad libitum in a research ward.

The resulting levels of nicotine are too low to be measured in plasma, but they can be measured in urine because concentrations of nicotine are much higher in urine than in plasma. In contrast, for cotinine, which is much less avidly taken up by tissue, the elimination half-lives derived from urine excretion data and plasma level data are similar.[14,29]

In conclusion, we provide novel data on minor alkaloid levels in tobacco products and in users of these products. Our data suggest that measurement of anabasine and anatabine levels in urine has the potential to be a biomarker of tobacco consumption and exposure to toxic substances in tobacco, even in individuals using nicotine-containing medications, since such medications do not contain these alkaloids. □

Contributors

P. Jacob and N. L. Benowitz designed the study. N. L. Benowitz was responsible for all experiments involving human subjects. P. Jacob, A. T. Shulgin, and L. Yu developed the chemical analytical methods for measuring concentrations of nicotine and other tobacco alkaloids in tobacco and in human biological fluids. L. Yu was responsible for overseeing the chemical analyses. All 4 authors guarantee the integrity of the research.

Acknowledgments

Funding provided by the Cigarette and Tobacco Surtax of the State of California through the Tobacco-Related Disease Research Program of the University of California (grant 4RT-0023) and the National Institute on Drug Abuse, National Institutes of Health (NIH) (grants DA02277 and DA01696) is gratefully acknowledged. Clinical studies were carried out in the General Clinical Research Center at San Francisco General Hospital Medical Center with the support of the Division of Research Resources, NIH (grant RR-00083).

We are grateful to Patricia Buley, Sandra Tinetti, and the staff of the General Clinical Research Center for assistance in conducting the clinical study; Gang Liang, Irving Fong, and Minjiang Duan for carrying out the gas chromatography–mass spectrometry analyses; Gunnard Modin for statistical analysis; and Kaye Welch for editorial assistance.

References

1. Gorrod JW, Wahren J, eds. *Nicotine and Related Alkaloids: Absorption, Distribution, Metabolism and Excretion*. London, England: Chapman & Hall; 1993.
2. Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. *Annu Rev Pharmacol Toxicol*. 1996;36:597–613.
3. Benowitz NL. Smoking-induced coronary vasoconstriction: implications for therapeutic use of nicotine. *J Am Coll Cardiol*. 1993;22: 648–649.
4. Clark MSG, Rand MJ, Vanov S. Comparison of pharmacological activity of nicotine and related alkaloids occurring in cigarette smoke. *Arch Int Pharmacodynamics*. 1965;156:363–379.
5. Nasirov SK, Ryabchenko VP, Khalikova FR, Khazbievich IS, Kashkova EK. Anabasine hydrochloride—a new antismoking agent. *Khimiko-Farmatsevticheskii Zhurnal*. 1978; 12:149–152.
6. Hoffman D, Hecht SS. Nicotine-derived N-nitrosamines and tobacco-related cancer: current status and future directions. *Cancer Res*. 1985;45:934–944.
7. Nguyen TL, Gruenke L, Castagnoli N Jr. Metabolic oxidation of nicotine to chemically reactive intermediates. *J Med Chem*. 1979;22: 259–263.
8. Bong HK, Shigenaga MK. Metabolism-dependent covalent binding of S(-)-3H-nicotine to lung microsomes in vitro. *Arch Pharmacol Res*. 1993;16:89–93.
9. Shigenaga MK, Kim BH, Caldera-Munoz P, et al. Liver and lung microsomal metabolism of the tobacco alkaloid β–nicotyrine. *Chem Res Toxicol*. 1989;2:282–287.
10. Benowitz NL. The use of biologic fluid samples in assessing smoke consumption. In: Grabowski J, Bell CS, eds. *Measurement in the Analysis and Treatment of Smoking Behavior*. Washington, DC: National Institute on Drug Abuse; 1984:6–26. NIDA Monograph 48.
11. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev*. 1996;18:188–204.
12. Sepkovic DW, Haley NJ. Biomedical applications of cotinine quantitation in smoking-related research. *Am J Public Health*. 1985;75: 663–664.
13. Galeazzi RL, Daenens P, Gugger M. Steady state concentrations of cotinine as a measure of nicotine intake by smokers. *Eur J Clin Pharmacol*. 1985;28:301–304.
14. Benowitz NL, Jacob P III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin Pharmacol Ther*. 1994;56: 483–493.
15. Schmeltz I, Hoffmann D. Nitrogen containing compounds in tobacco and tobacco smoke. *Chem Rev*. 1977;77:295–311.
16. Jacob P III, Benowitz NL. Oxidative metabolism of nicotine in vivo. In: Adlkofer F, Thurau K, eds. *Effects of Nicotine on Biological Systems*. Basel, Switzerland: Birkhauser Verlag; 1991:35–44.
17. Benowitz NL, Jacob P III, Denaro C, Jenkins R. Stable isotope studies of nicotine kinetics and bioavailability. *Clin Pharmacol Ther*. 1991;49: 270–277.
18. Jacob P III, Yu L, Wilson M, Benowitz NL. Selected ion monitoring method for determination of nicotine, cotinine, and deuterium-labeled analogs. Absence of an isotope effect in the clearance of (S)-nicotine-3'-3'-d_2 in humans. *Biol Mass Spectrom*. 1991;20:247–252.

19. Jacob P III, Yu L, Liang G, Shulgin AT, Benowitz NL. Gas chromatographic-mass spectrometric method for determination of anabasine, anatabine and other tobacco alkaloids in urine of smokers and smokeless tobacco users. *J Chromatogr Biomed Applications*. 1993;619: 49–61.
20. Benowitz NL, Hall SM, Herning RI, Jacob P III, Jones RT, Osman AL. Smokers of low yield cigarettes do not consume less nicotine. *N Engl J Med*. 1983;309:139–142.
21. Andersen RA, Burton HR, Fleming PD, Hamilton-Kemp TR. Effect of storage conditions on nitrosated, acylated, and oxidized pyridine alkaloid derivatives in smokeless tobacco products. *Cancer Res*. 1989;49:5895–5900.
22. Benowitz NL, Jacob P III. Daily intake of nicotine during cigarette smoking. *Clin Pharmacol Ther*. 1984;35:499–504.
23. Benowitz NL, Jacob P III, Kozlowski L, Yu L. Influence of smoking fewer cigarettes on exposure to tar, nicotine, and carbon monoxide. *N Engl J Med*. 1986;314:1310–1313.
24. Benowitz NL, Jacob P III, Yu L. Daily use of smokeless tobacco: systemic effects. *Ann Intern Med*. 1989;111:112–116.
25. Armitage AK, Dollery CT, Houseman TH, Kohner E, Lewis PJ, Turner DM. Absorption of nicotine from small cigars. *Clin Pharmacol Ther*. 1978;23:143–151.
26. Turner JAM, Sillett RW, McNicol MW. Effect of cigar smoking on carboxyhaemoglobin and plasma nicotine concentrations in primary pipe and cigar smokers and ex-cigarette smokers. *BMJ*. 1977;2:1387–1389.
27. Wald NJ, Idle M, Boreham J, Bailey A, Van Vunakis H. Serum cotinine levels in pipe smokers: evidence against nicotine as cause of coronary heart disease. *Lancet*. 1981;2:775–777.
28. Beckett AH, Gorrod JW, Jenner P. A possible relation between pka1 and lipid solubility and the amounts excreted in urine of some tobacco alkaloids given to man. *J Pharm Pharmacol*. 1972;24:115–120.
29. Benowitz NL, Kuyt F, Jacob P III, Jones RT, Osman AL. Cotinine disposition and effects. *Clin Pharmacol Ther*. 1983;309:139–142.

Managed Care in American Indian and Alaska Native Communities

Mim Dixon

This book will help American Indian and Alaska Native peoples understand managed care and the opportunities and challenges presented to their communities, as well as help health care professionals in managed care better understand their perspectives and goals. The examples discussed are in the context of Indian health care systems, but they will provide insight not only for those working inside this community, but also in other minority health systems.

1998 • softcover • 195 pages • Stock no. 0-87553-238-1/INAD99

$7.00 APHA members* • $10.00 Nonmembers
(add shipping and handling costs to all orders)

*APHA members may purchase up to 2 copies at this price

New from the American Public Health Association!

APHA

American Public Health Association Publications Sales
PO Box 753
Waldorf, MD 20604-0753.
Voice: (301) 893-1894; Fax: (301) 843-0159
E-mail: TASCO1@APHA.ORG; Web: www.apha.org